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Isolating humic-like substances from olive oil milling waste into a soluble bio organic (SBO) product

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ORRE, SARA:

Isolating humic-like substances from olive oil milling waste and turning them into a soluble bio organic (SBO) product

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ABSTRACT

This thesis was done during Erasmus+ exchange program in Polytechnic University of Valencia, Campus of Alcoy. The thesis was inspired by the research done in The Department of Chemistry of the University of Torino where a soluble bio-based (SBO) product used as a chemical auxiliary in chemical processes was successfully prepared from urban bio wastes using certain digestion and oven treatment. According to several studies humic-like substances and SBO have many similar chemical properties. The aim of this study was to isolate humic-like substances from hazardous olive oil milling waste called alperujo, and to examine if the waste is suitable to be turned into an SBO-product using the same methods as used in Italy.

The sample was humid alperujo collected from a two-phase centrifugation process from olive oil milling plant located in Millena, southern Spain. The sample was digested in 64ºC, 4 hours with pH 13, filtered with fabric filters and membranes, and finally put in the oven in 40 ºC for 24 hours. The sample was characterized after every membrane filtration using 3 commercial SBO-products as reference results.

According to the characterization, the composition of the final Millena SBO was close to the commercial SBO-products. The function of the Millena SBO was tested in normally acidic conditions requiring a waste water treatment method called photo-Fenton reaction with pH 5. A solution containing 6 different contaminants was prepared to be purified in the reaction. Because of the photoactive properties of humic-like substances, the reaction was possible to carry on on neutral, pH and the contaminant solution was purified with good results besides one contaminant. According to the results alperujo suits well to be an SBO-product, and the topic should be further researched.

Key words: olive oil, biowaste, alperujo, humic-like substances, SBO, photo-Fenton reaction

Lahden ammattikorkeakoulu
Koulutusohjelma

ORRE, SARA:

Humiinipitoisten aineiden erottaminen oliiviöljyn prosessijätteestä ja niiden kehitys liukoiseksi bio-orgaaniseksi (SBO) tuotteeksi

Energia- ja ympäristöteknologian opinnäytetyö, 71 sivua

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TIIVISTELMÄ

Tämä opinnäytetyö tehtiin Valencian polyteknisen yliopiston Alcoyn campuksella Erasmus+ -opiskelijavaihdon aikana. Työ jatkaa Torinon yliopiston kemian osastolla aloitettua tutkimusta, jossa kaupunkibiojätteestä tehtiin bio-pohjainen tuote (SBO) apuvälineeksi erilaisiin kemiallisiin prosesseihin hyödyntäen uuttoa ja uunikäsittelyä. Tutkimusten mukaan humiinisubstansseilla ja SBO:lla on monia samankaltaisia kemiallisia ominaisuuksia, joten tämän työn tavoitteena oli erottaa humiinisubstanssit oliiviöljyn prosessijätteestä ja tutkia, onko jätteestä mahdollista saada aikaan SBO-tuote samoin menetelmin kuin Italiassa kaupunkibiojätteestä.

Oliiviöljyn prosessijätettä kutsutaan alperujoksi, ja se on luokiteltu haitalliseksi jätteeksi. Työssä käytettävä alperujo oli peräisin oliiviöljyn tuotannon kaksivaiheisesta sentrifugointiprosessista. Näyte kerättiin oliiviöljyn tuotantolaitokselta, joka sijaitsee Millenassa Etelä-Espanjassa. Työn ensimmäisessä vaiheessa humiinisubstanssit erotettiin alperujosta uuttamalla näytettä ensin 64 asteen lämpötilassa 4 tuntia pH:n ollessa 13. Tämän jälkeen näyteliuos suodatettiin kangassuodattimilla ja membraanikalvoilla ja laitettiin uuniin 40 asteen lämpötilaan 24 tunniksi. Näyte karakterisoitiin jokaisen membraanikalvoilla tehdyn suodatuksen jälkeen käyttäen viitetuloksina kolmea kaupallista SBO-tuotetta.

Karakterisointi osoitti lopullisen Millena SBO-tuotteen olevan samankaltainen kemiallisilta ominaisuuksiltaan kaupallisten tuotteiden kanssa. Työn toisessa vaiheessa Millena SBO:n toiminta testattiin foto-Fenton-reaktiolla, jota käytetään jäteveden puhdistukseen. Reaktio vaatii normaalisti happamat olosuhteet, mutta SBO-tuotteen avulla reaktio oli mahdollista suorittaa neutraalilla pH:lla, mikä johtuu humiinisubstanssien fotoaktiivisista ominaisuuksista. Reaktiota varten valmistettiin liuos, joka sisälsi 6 haitta-ainetta. Foto-Fenton-reaktio kykeni poistamaan viisi haitta-ainetta liuoksesta hyvin tuloksin, kun reaktioon lisättiin SBO-tuotetta pH:n ollessa 5. Tulosten perusteella alperujo soveltuu hyvin SBO-tuotteeksi, ja aihe on jatkotutkimuksen arvoinen.

Asiasanat: oliiviöljy, biojäte, alperujo, humiinisubstanssit, SBO, foto-Fenton-reaktio

TABLE OF CONTENTS

Glossary

SBO – Soluble bio organic

SBO Millena – SBO made from alperujo from Millena

HA – Humic acid

FA – Fulvic acid

HPLC – High performance liquid chromatography

UHPLC – Ultra high performance liquid chromatography

KOH – Potassium hydroxide

R – Retained solution

F – Filtered solution

OMW - Olive milling wastewater

DOM – Dissolved organic matter

1 INTRODUCTION

Waste management is one of the challenges the world is facing today and even more in the future due to the population growth. The more people are inhabiting the planet, the more it has to offer its natural resources which are turned into essential products for living such as materials, energy and food. Finally, it all comes back around as waste. According to EU's waste hierarchy, the first thing to do would be preventing generating waste in the first place, the second best option is to prepare the waste for re-use, the third best option is to recycle the waste, the fourth best option is to turn the waste into energy in incineration plants, and finally the worst alternative is to dump the waste to landfills. Landfills have a seriously problematic nature because they emit methane, a greenhouse gas into the air. When leaking they also can release chemicals into the groundwater and soil and that way pollute the environment and become a health risk for humans and do some serious harm to the ecosystems of nature. The EU is trying to minimize landfilling in all possible ways: it is strickening the land filling legislation and has closed thousands of land filling sites. (European union. 2010)

A disconserning fact considering the problematic effects of landfilling is the amount of bio wastes going to landfills. Still 40% of biodegradable garden waste, food waste and alike waste from food processing plants were put in landfills in 2010. Fortunately, the potential of bio waste as a renewable energy source has been noticed, and the production of bio gas and bio fuel have become more common. According to EU's strategies, the number of bio waste gone in landfills should decrease to 35% in 2016. EU also estimates that it is possible to achieve one-third of the EU's 2020 goal for renewable energy use in transport by using bio waste turned into bio gas. (European union. 2010)

Increasing bio waste use in renewable energy production is a good way to dispose it, and, at the same time, decrease the use of fossil fuels. It still does not take advantage of all the waste generated and a huge part of it still goes to landfills. The amount of bio waste will increase in the future

due to the population growth so the new ways to exploit it are valuable when facing the challenges of the future.

According to a project called Biochemenergy (Montoneri. 2010) biowaste can be used to produce chemical auxiliaries and materials for the usage of chemical industry. The project was about collecting urban bio waste and turning it into a soluble bio organic (SBO) product. SBO has some special chemical properties, i.e. because of its surfactant properties SBO can be used in textile dyeing and manufacturing detergents.

According to many studies, the properties and chemical composition of SBO are really close to humic substances. Humic substances are known to be e.g. photoactive. The photoactivity of SBO produced in Biochemergy project has been previously studied by investigating if it can make a waste water treatment technique photo-Fenton reaction to work more environmentally friendly (Gomis et al. 2014).

This project was done during Erasmus+ -student exchange program in the Polytechnic University of Valencia, Campus of Alcoy. Campus of Alcoy has been a part of previously described studies and this project continues the research of the biowaste usage in chemical industry. The goal of this project was to prepare SBO from olive oil milling waste called alperujo using the same method that was performed during Biochemenergy project preparing SBO from urban bio waste. Alperujo was chosen to be the target of this project because it is hazardous waste which is difficult to dispose of and huge amounts are produced in Spain every year. The sample of alperujo was collected from olive oil milling plant from a town called Millena, located in southern Spain.

The function of SBO produced from alperujo is tested with photo-Fenton reaction imitating the method of J. Gomis' work which was performed with Italian SBO in 2014 in the laboratory of Campus of Alcoy. The goal is to get the reaction which normally works only on acidic conditions to work with neutral pH by adding SBO into the reaction along with iron. If this succees, a new chemical auxiliary is created, the process will be more

environmental friendly, and there is a new way to dispose hazardous alperujo.

This project was an experiment – the goal was to find out if these known methods used with urban bio waste work also with alperujo. The research continues after this project and different methods will be tested to obtain the optimum method for turning alperujo into SBO.

This project was carried out in the laboratory of Campus of Alcoy. Laboratory work was performed together with Álvaro Francés Lozano under a guidance of Sara García Ballesteros and Antonio Arques.

2 THEORY

The first chapter of the theory part contains information about the olive oil production in Spain and the waste called alperujo, which is generated in the production and used in this project. The second chapter goes in alperujo a little deeper on molecular level – it describes the nature of humic substances and why they can be used to generate SBO. The last chapter is about photo-Fenton reaction, which is used to test the function of the SBO-product of this project.

2.1 Olive oil milling in Spain

In Mediterranean countries, olive oil has been a part of every-day diet for many generations. Nowadays, the usage of olive oil is common all over the world because of its healthy and nutritional properties. In 2011 Spain exported annually 840 000 tons of olive oil and the number is increasing every year. The biggest markets are inside EU and in third countries such as USA, Brazil, Japan, Australia, Russia and China. (Agriculture and Rural Development on EUROPA. 2016)

The most part of the world's olive oil production takes place in the EU mostly because of the three leading countries. The EU products 73% of the world's olive oil of which Spain, Italy and Greece product 97%. Spain is the world's and, at the same time, EU's biggest manufacturer with the share of 62% of the EU's generation. The production is predicted to increase with average yield season to 1,677 million tons by year 2022 (Figure 1). (Agriculture and Rural Development on EUROPA. 2012)

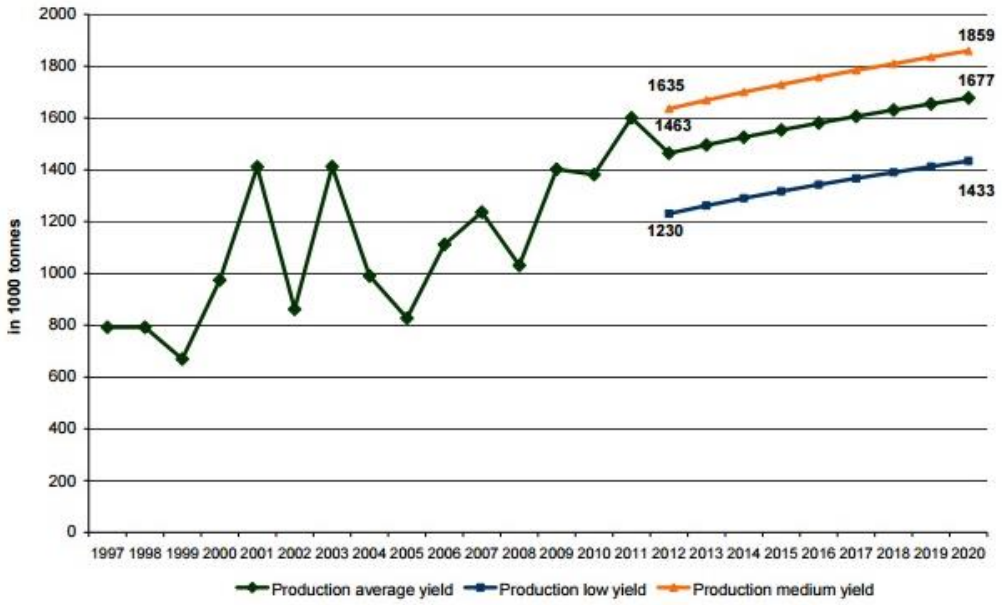


Figure 1 Prediction of olive oil production (Agriculture and Rural Development on EUROPA 2012)

2.1.1 Milling process

In the last few decades, olive oil milling procedures' waste treatment have faced a revolution when oil producers have little by little abjured the old three-phase centrifugation technology, and taken a new method called two-phase centrifugation in its place.

The three-phase centrifugation system (Figure 2) was introduced in the beginning of seventies. With this method, it was possible to get the solid part of a olive called ''olive cake'' or ''orujo'' and the olive milling vegetative water separately from the process. This method was quite problematic in the winter time from November to February when Mediterranean countries have their rainy season. In these months the vegetation water is mixed up with a huge amounts of water. Water gets into the process at the phases of washing, centrifugation and oil washing/recovery of the oil in liquid fraction. Water with the vegetation water generate the olive milling wastewater (OMW) which is very pollutious and challenging to disposal. Olive oil milling industry has succeeded to invent a few ways to do this –

OMW has been used in agriculture as fertilizers and it has been stored in evaporation pounds. (Alburquerque et al. 2004, 195-196)

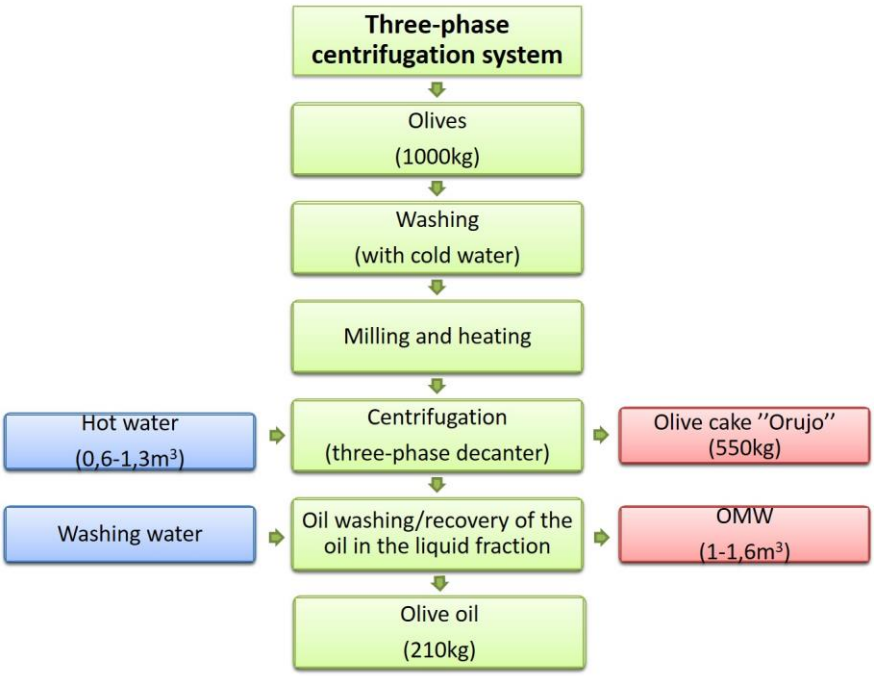


Figure 2 Three-phase centrifugation system (Alburquerque et al. 2004, 196)

Despite of these disposal methods, there was some need for a new method, which would make an improvement to the waste fractions. In early ninety century, a two-phase centrifugation olive oil producing technology started to spread and replace the old three-phase centrifugation systems. The biggest difference between the three-phase and the two-phase systems was the amount of OMW – it dropped from 1-1,6m^2 to 0,2m^2 when two-phase centrifugation system was put into operation (Figure 3). It also used less water and energy than the old system. According to these changes, the new centrifugation technology was regarded ecological, and it started to become common rapidly all over Spain. In 2004, it was estimated that approximately 90% of olive mills run with the two-phase system. Instead of causing huge OMW number, this method does produce bigger amount of very humid solid waste called ''alperujo''. As seen in Figure 3, processing 1000 kg of olives the two-phased centrifugation

generates 800 kg of alperujo. With Spanish production volumes it means that the yearly yield of alperujo can reach approximately four million tons. (Alburquerque et al. 2004, 196)

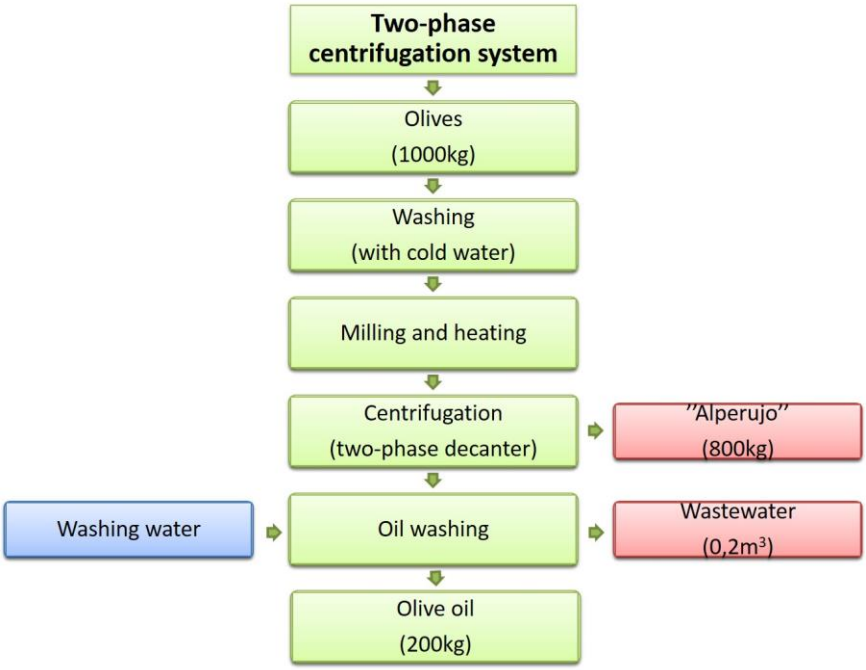


Figure 3 Two-phase centrifugation system (Alburquerque et al. 2004, 196)

2.1.2 Alperujo

Alperujo is a wet solid olive oil milling waste. It is also acidic so it is classified as hazardous waste. Alperujo has been characterized to have a rich content in organic matter, containing mainly lignin, hemicellulose and cellulose but also fats, hydrosoluble carbohydrates and proteins. (Alburquerque et al. 2004. 199)

There are some ways to dispose alperujo – e.g. it can be transformed into another co-product called orujillo. To get orujillo, the residual oil of alperujo must first be extracted by second centrifugation, dried and treated with hexane in chemical extraction. The problem of this method is the drying phase, which is noted to generate polycyclic aromatic hydrocarbons (PAH)

to orujillo. Because of this, the method requires another purification step which increases the production costs. It is also possible to use alperujo as a fertilizer, but it needs to get composted before treating the soil with it, because it includes phenol, organic and fatty acid contents. These substances make alperujo to have phytotoxic and antimicrobial effects which are bad for the soil. (Alburquerque et al. 2004. 196)

2.2 Humic-like substances

Humic-like substances are a part of dissolved organic matter (DOM). Stevenson has been studying humic-like substances from this point of view. He focuses on the constitution of soil organic matter by dividing it to five different pools. Each of them has their own meanings and tasks in the soil dynamics and nutrient flows. First on the soil surface lays the litter, which is a macro-organic material and has a great purpose on the task of cycling nutrients. The next pool is the ‘’light’’ fraction which consists of decomposting plant residues. The purpose of the ‘’light’’ fraction is to nourish the plants which are growing on the ground. Micro-organisms living in the soil form the pool of microbial biomass. It has a huge role in decomposition of the plant residues. The fourth pool consists of the organics which are soluble in water. The last pool is the stable humus which forms a bulk of agricultural soils. Definitions of stable humus vary a lot but here it includes all the organic substances excluded ‘’light’’ fraction. Finally the pool of stable humus can be divided into groups of humic substances and non-humic substances. (Stevenson 1994, 1-6)

There are three kind of humic substances: humic acids, fulvic acids and humin. Humic and fulvic acids are soluble with certain solvents and they can be exracted from soil. Humic acids can be exracted by dilute alkali and other reagents whereas fulvic acids by dilute alkali and dilute acid. Humus is not soluble in either of alkalis and because of this, dilutions with alkali or acid are used to separate the acids from humin. (Stevenson 1994, 33)

Humic substances show up in many forms of matter all over the nature. For example, humic acid appears in solid or colloidal form in soils,

wetlands and freshwater. In addition solid forms exist in lignite and coals, and also dissolved in freshwater. (Killops and Killops 1993, according to Steinberg 2003, 12)

Researchers have been studying humic substances for many decades, but their generation and chemical composition is still partly unclear. The reason why researching keeps going on and on is their unique chemical properties – for example many studies have evidenced that they are photochemically active (Tipping 2005, 1). There are some theories about their purpose in the nature's circulation: e.g example Davies and Ghabbour describe humic substances as a product of death. They are the link in the twilight zone between the life and the death of a plant – in the phase where the brown colour starts to evade the plant's area. (Davies & Ghabbour. 11)

2.2.1 Chemical composition of humic substances

According to Stevenson, humus may contain all the known biochemical compounds that are synthesized by living organisms. These well-known compounds make the group of non-humic substances. The known compounds consist of amino acids, carbohydrates, fats and other organic substituents etc. Humic substances' characterization is more complicated. Generally they are described to be high oxygen-containing functional groups like COOH, phenolic and/or enolic OH, alcoholic OH and C=O of quinoes. These functional groups can be seen in model structure of humic acid (Figure 4). Humic substances are defined to be coloured yellow to black and have a comparable high molecular weight. They are formed in secondary synthesis reactions. (Stevenson 1994, 33)

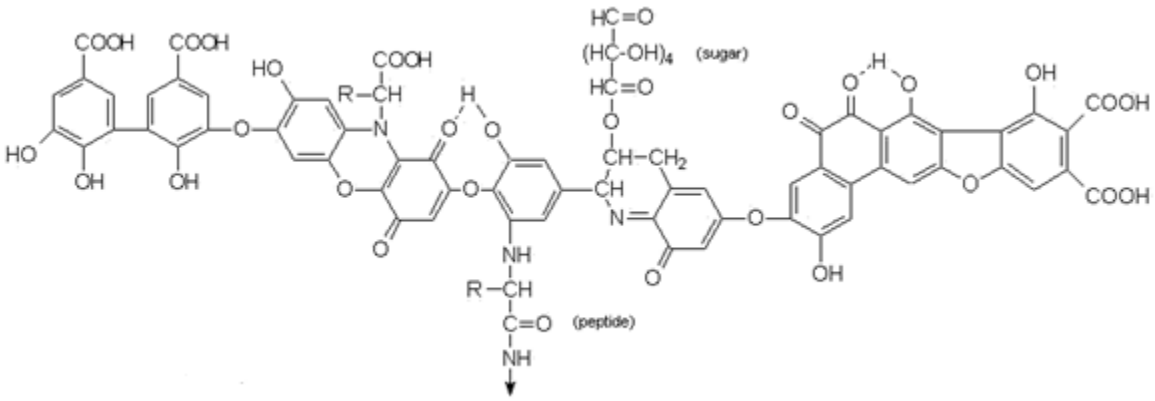


Figure 4 Chemical structure of humic acid (Stevenson 1994)

2.2.2 Photo-activity of humic substances

Dissolved organic carbon, which can be found from natural waters consists mostly of humic substances. This matter plays an important part in water's photochemistry, because it absorbs sun's radiation. Figure 5 presents UV-Vis absorption spectra for three different humic acids: fulvic acid from Suwannee river, Leonardite humic acid and Pahokee humic acid from peat. There is also a simplified solar spectrum for water containing all these humic substances. According to the Figure 5, the most absorption happens between 300-700 nm. This means that humic substances primary excitation step needs to be 95-41 $kcal^{-1}$. The same amount of energy is able to produce many different photochemical processes. That is the reason why they can be used as an agent in chemical processes including this study's photo-Fenton reaction (Montoneri et al. 2013. 79-80). They can assist the photo-Fenton reaction because of their ability to generate higly reactive species under sun's radiation. (Gomis et al. 2014. 177)

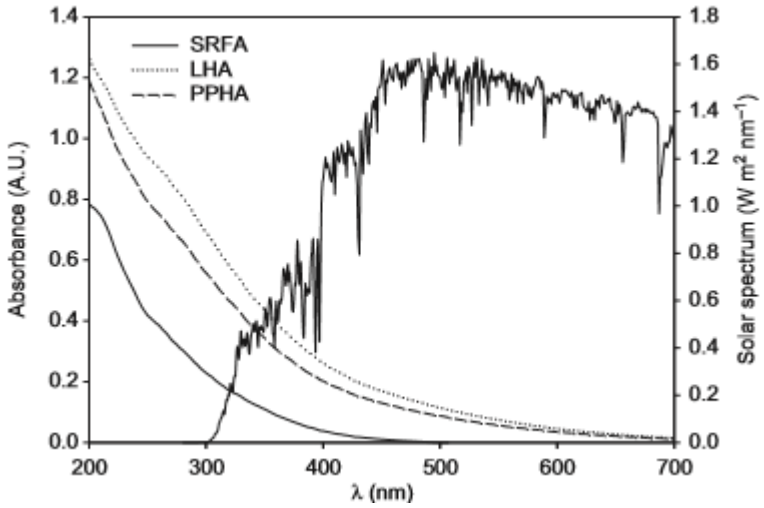


Figure 5 Solar spectrum and UV-vis absorption spectra for SRFA=Suwannee River Fulvic acid; LHA=Leonardite humic acid; PPHA=Pahokee peat humic acid (Montoneri et al. 2013)

2.3 Photo-Fenton reaction

This Chapter begins describing advanced oxidation processes (AOP) on the basic level. The next section contains more information about different Fenton-reactions. For this study, the most important reaction is the photo-Fenton reaction which is used to test if SBO can make it function with neutral pH.

2.3.1 Advanced oxidation processes

Advanced oxidation processes (AOP) are highly effective methods for waste water treatment. AOPs are common methods to treat industry waste waters but they haven't been used to treat municipal waste waters yet. (Suomen Vesilaitosyhdistys ry, 2016, 25). Generally, operation of oxidation processes is based on their ability to mineralize the most harmful organic contaminants into harmless products, e.g. into water and inorganics. The difference between normal oxidation processes and AOP's is their ability to produce highly reactive hydroxyl (OH) radicals. (Andreozzia et al. 1999, 52)

Hydroxyl radicals are one of the most reactive oxidants. Figure 6 compares some common oxidants about their standard oxidation potential and relative strength referencing to chlorine (1) (Siegrist et al. 2001). Hydroxyl radical is the most effective among the common oxidants, having the oxidation potential 2,8 volts and relative strength 2,0. They are especially suitable for waste water treatment because of their little selectivity – it means they can clean the water from pollutants compherensively (Andreozzia et al. 1999, 52).

Chemical species	Standard oxidation potential (volts)	Relative strength (chlorine = 1)
Hydroxyl radical ($OH^{-\bullet}$)*	2.8	2.0
Sulfate radical ($SO_4^{-\bullet}$)	2.5	1.8
Ozone	2.1	1.5
Sodium persulfate	2.0	1.5
Hydrogen peroxide	1.8	1.3
Permanganate (Na/K)	1.7	1.2
Chlorine	1.4	1.0
Oxygen	1.2	0.9
Superoxide ion ($O^{-\bullet}$)*	-2.4	-1.8

Figure 6 Oxidant strenghts (* can be formed when ozone and H_2O_2 decompose) (Siegrist et al. 2001)

AOPs offer many options for producing hydroxyl radicals:

- Combining different oxidants, catalysts and irradiation
- Using Fenton's reagent $(Fe(II)/H_2O_2)$
- UV/H_2O_2
- Ozonation
- hv/TiO_2
- Wet air oxidation
- Sonolysis
- Combinations of 2 or more of these methods (Hann et al. 2002, 94)

The production method should be taken into account every time when treating wastewater, because some methods suit better with certain waste water treatment methods than others. It is also important that the expensive reactants, like hydrogen peroxide (H_2O_2), are consumed cost-effectively. (Andreozzia et al. 1999, 52)

2.3.2 Fenton's reactions

Fenton's reaction uses hydrogen peroxide (H_2O_2) as an oxidant. H_2O_2 is not strong enough to work on it's own in this process, so H.J.H Fenton invented a reaction where H_2O_2 is reacting with iron at pH 2,5-3,5. Ferrous iron (II) is oxidized to ferric iron (III) by H_2O_2 – this reaction causes the generation of hydroxyl radicals which makes the reaction to oxidize pollutants effectively. As seen in Figure 6, oxidation potential gains from 1,8 V (H_2O_2) to 2,8 V ($HO\cdot$) because of the addition of iron. What makes the reaction long standing is the pH: when it is 2,5-3,5, iron (III) is reduced back to iron (II) and again oxidized by hydrogen peroxide to iron (III). The process keeps on running and the generation of hydroxyl radicals continuing. The chemical formula of the reaction is following:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \quad (1)$$

As described below, an ideal pH for Fenton's reaction is 2,5-3,5. If it is higher the iron doesn't work as a catalyst anymore. This is a problem especially for the environment: a low pH can cause metals to dissolve and make them end up in the natural waters. (ITRC 2005, 10-12)

The principle of photo-Fenton reaction is the same than normal Fenton-reaction's except it is enhanced with a light source, e.g. UV-light, as seen in reaction's chemical formula (3). Adding light makes photo-Fenton reaction degrading pollutants effectively, because it generates an additional hydroxyl radical. The reaction starts with normal Fenton's reaction (2) but with light, the Fe^{3+} reduces photocatalytically to Fe^{2+}. This reaction generates an additional hydroxyl radical. Finally generated

hydroxyl radicals oxidize organic species (RH) i.e. the contaminants of the waste water are degrading. (4) (Moraes et al. 2004. 1183)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\cdot} \quad (2)$$

$$Fe^{3+} + H_2O \overset{hv}{\rightarrow} Fe^{2+} + H^+ + HO^{\cdot} \quad (3)$$

$$HO^{\cdot} + RH \rightarrow H_2O + R^{\cdot} \quad (4)$$

2.4 Using SBO to make photo-Fenton neutral media

Humic substances can be used to prepare SBO because of their similar chemical properties. In general, SBO can be described as a mixture of macromolecules very similar with humic substances, and their molecular weight varying from 67 to 463 kg mol-1. (Gomis et al. 2014. 177)

SBO contains many minerals which are difficult to cut off from organic matter, one of these minerals is iron. Iron is also an essential chemical in the photo-Fenton reaction. Iron ions are not normally soluble in neutral pH, this is the reason why photo-Fenton reaction works most effective under pH 2,5-3,5. When SBO is added in the reaction, it is possible to get it function with neutral pH, due to the iron ions included in SBO in addition to its photochemical properties. (Gomis et al. 2013)

3 ANALYTICAL METHODS, EQUIPMENT AND TECHNIQUES

This chapter includes the basic information of the analytical methods and equipment used in this project. Analysis were needed to characterize the sample of alperujo between membrane filtrations to find out when the composition was the most optimal to be a new SBO-product. Analysis were also needed during the photo-Fenton reaction. Chapter also contains information about the most used techniques e.g membrane filtration.

3.1 Membrane filtration

Membrane filtration technology is an effective and diverse method for separation, concentration and purification. The technology made a huge breakthrough in the middle of 1900-century when it was used first time in an industrial application. Its usage has become common in many industrial fields ever since. Membrane filtration is used in many areas including water and waste water purification, gas separation, pollution control, hemodialysis, proteins and microorganism separation etc. Even though it's very versatile and effective method at the moment, it will be constantly developed so it can face the challenges of the future. (Wang et al. 2010. 2.)

Because the membrane technology is utilized in applications, there are also a wide cathegory of membranes with different properties (Figure 7). Their shape can vary from flat sheets to long capillary membranes. Their driving force usually in waste water treatment is pressure, but it can also be temperature, concentration or voltage. In turn their structure can be solid, porous or charged film with symmetric or asymmetric film. Membranes can filter matter in many forms and shapes: liquids, gases, particles, molecules and ions. (Wang et al. 2010. 3)

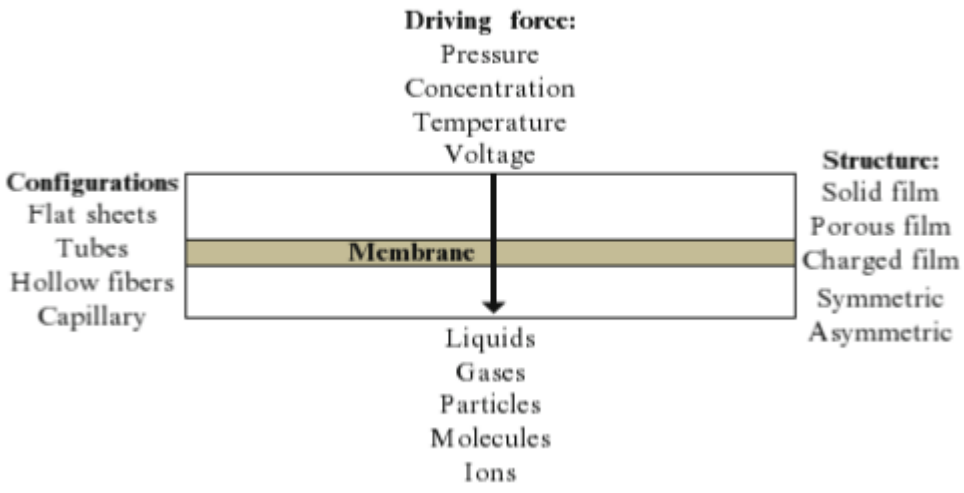


Figure 7 Possible features of membranes (Wang et al. 2010)

When using pressure as a driving force, there are four membrane sizes to choose which are listed from biggest to smallest: microfiltration (50-10^4nm), ultrafiltration (5-100nm), nanofiltration (1-10nm) and reverse osmosis (<2nm). Figure 8 presents how certain sized molecules go through each membrane, whereas molecules which are bigger than membrane pore size cannot pass through. The filtration method for every target can be chosen by the pore size of the membrane, for example micro filtration can be used to separate large colloids whereas ultrafiltration is needed when filtering macro molecules. (Wang et al. 2010. 4)

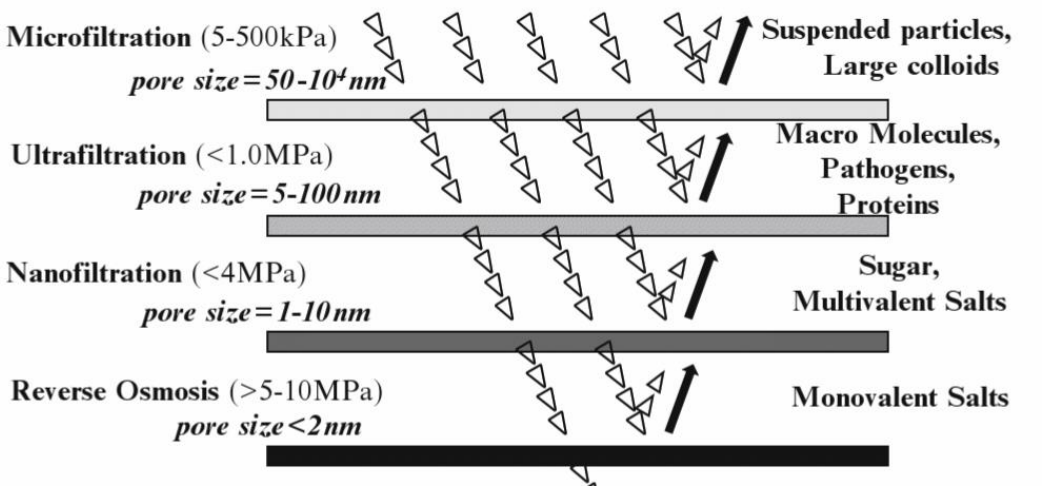
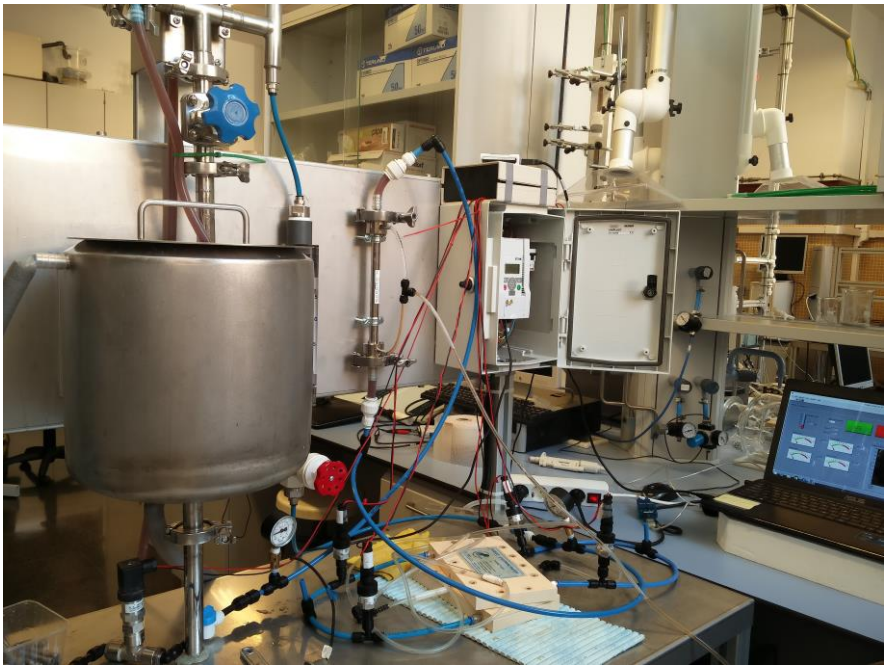


Figure 8 Filtration sizes (Wang et al. 2010)

The membrane filtration of this project was performed using membranes of Tami-Industries Valisette laboratory test kit (Picture 1). The kit includes membranes for micro filtration, ultra filtration and fine-ultrafiltration. The membranes are ceramic membranes with tubular shape. They function by separating molecules with their active layer which has a huge number of holes on it. (Tami-industries. 2016)

Picture 1 Tami-Industries Valisette laboratory test kit

The membrane filtration machine was built as a part of a doctoral thesis in Campus of Alcoy (Picture 2). The machine was used to separate molecules of humic substances from the sample solution. The membrane process divided the solution in filtrated (F) and retained (R) parts. The filtrated liquid consisted of molecules which went through the membrane, and the retained liquid molecules which were larger than the holes of the membrane and couldn’t pass through.



Picture 2 The machine for membrane filtration

3.2 High performance liquid chromatograph (HPLC)

Today high performance liquid chromatography (HPLC) is one of the most used methods to analyze and separate compounds in analytical chemistry. The principle diagram of HPLC is presented in Figure 9. First a sample wanted to get analyzed is put to an injector by syringe. Then it is forced to flow in a mobile phase through a column which is called a stationary phase. Typical solvents for the mobile phase are water, methanol, hexane, diethyl ether etc. The column and stationary phase are typically made from silica, but they can be also made from C8, cyanopropyl etc. The analysis can be modified to fit better for different substances by using different mobile and stationary phases. The separation of compounds happens in the column. Next compounds are detected by an ultra-violet detector and a diagram appears on a screen of a computer. The detection is based on the interaction of solvent between mobile and stationary phases. (Prichard et al. 2003. 1-2, 6, 11)

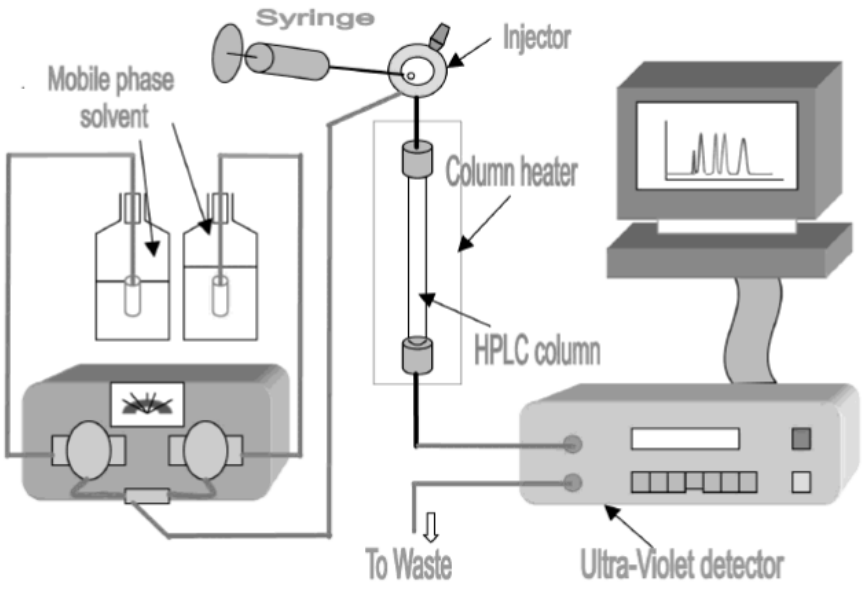
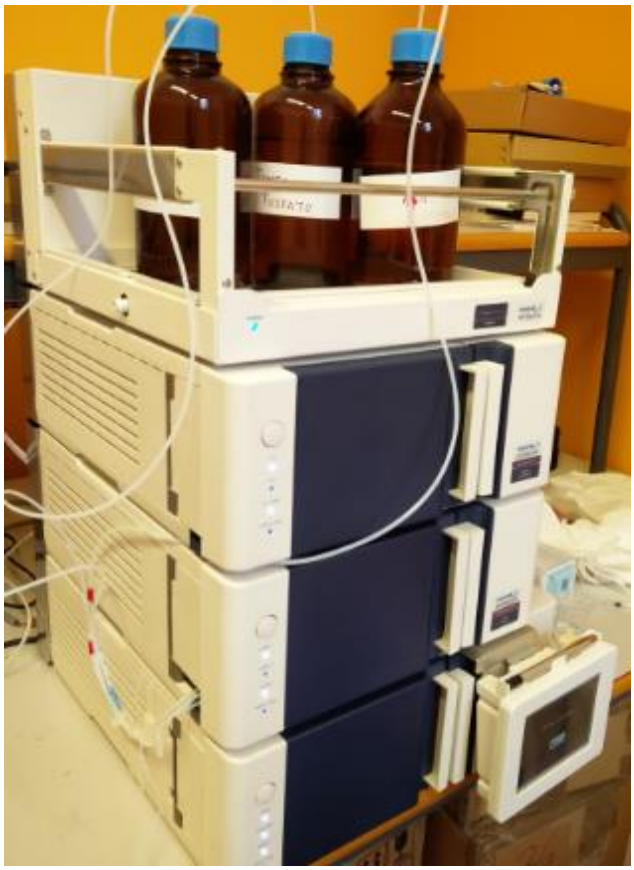


Figure 9 Principle of HPLC (Prichard et al. 2003)

Analysis of this project were performed with Hitachi HPLC liquid chromatograph (Picture 3). The system consists of Hitachi CM 5110 pump, Hitachi CM 5310 column oven and Hitachi CM 5420 UV-VIS detector.



Picture 3 Hitachi HTCM liquid chromatograph

3.3 UV-Vis Spectrophotometer

UV-Vis spectrophotometer technology is based on measuring radiation which is absorbed by different compounds of a matter. Every matter absorbs radiation by its characteristic way on specific wavelengths. Absorbation happens, when electrons are moving from lower orbital to higher levels because of the certain wavelengths. By knowing the wavelength and absorbtion of examined matter, it is possible to measure whether the sample contains the matter or not. (Higson. 2003. 112)

Spectrophotometers can be divided into two functional groups: single-beam spectrophotometers which use single light beam to generate the radiation, and double-beam spectrophotometers which use two light beams for the same purpose (Higson. 2003. 120-121). This study's

analysis were performed with Hitachi Double Beam Spectrophotometer UH5300 (Picture 4).

Picture 4 Hitachi Double Beam spectrophotometer UH5300

The principle of a double-beam spectrophotometer is presented in Figure 10. The technique uses a light source, usually Tungsten filament lamps, to generate two radiation beams with the same intensity. Both beams go through a monochromator which lets only the desired wavelengths to pass and radiate the sample and reference solvents. Therefore, with the monochromator, it is possible to select a range of wavelengths to the measurement. At next phase both radiations, which have gone through the sample and reference solvents, are detected by photo-multiplyer detectors. As a result there are two spectras: one from the reference solvent which is called a baseline spectra and one from the sample solvent. The detectors record and substract the baseline spectra from the sample solvent spectra. Finally the correct spectra can be read from a

computer. (Higson. 2003. 115-122)

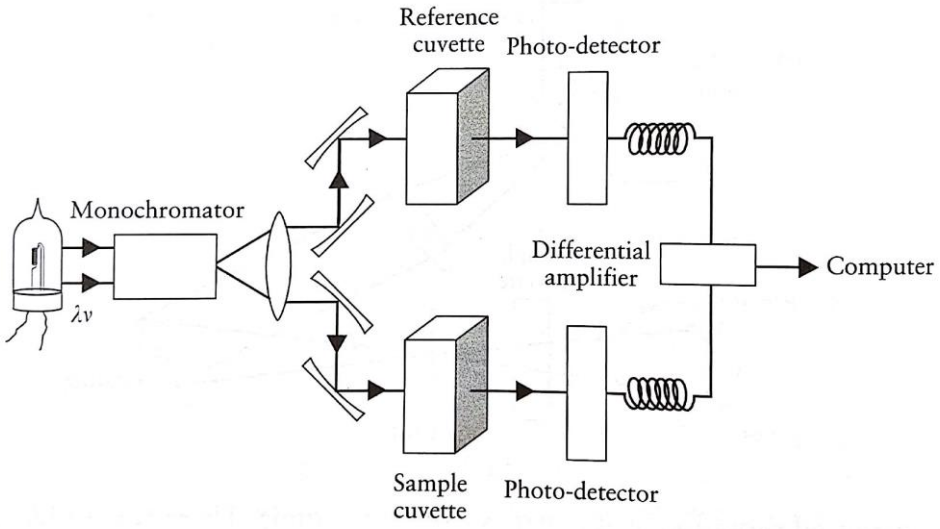


Figure 10 Principle of double-beam spectrophotometer (Higson. 2003)

3.4 TOC/TN analyzer

Total organic carbon (TOC) and total nitrogen (TN) were measured with a TOC/TN analyzer which uses a combustion catalytic oxidation method combined with a NDIR method (infrared gas analyzer). The analyzer heats samples to 680°C in a combustion furnace in oxygen rich environment with a platinum catalyst. Total carbon (TC) is decomposed and turned into CO^2, which is detected by the NDIR method. Next the oxidized sample goes through a sparking process where inorganic carbon (IC) turns to CO^2 and is detected again with the NDIR method. Finally TOC is calculated by substracting TC from IC. (Figure 11) (Simadzu Corporation. 2016)

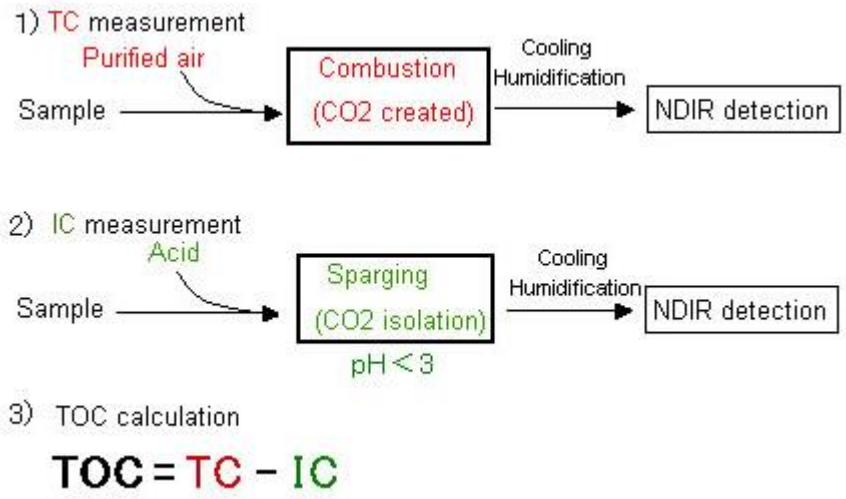
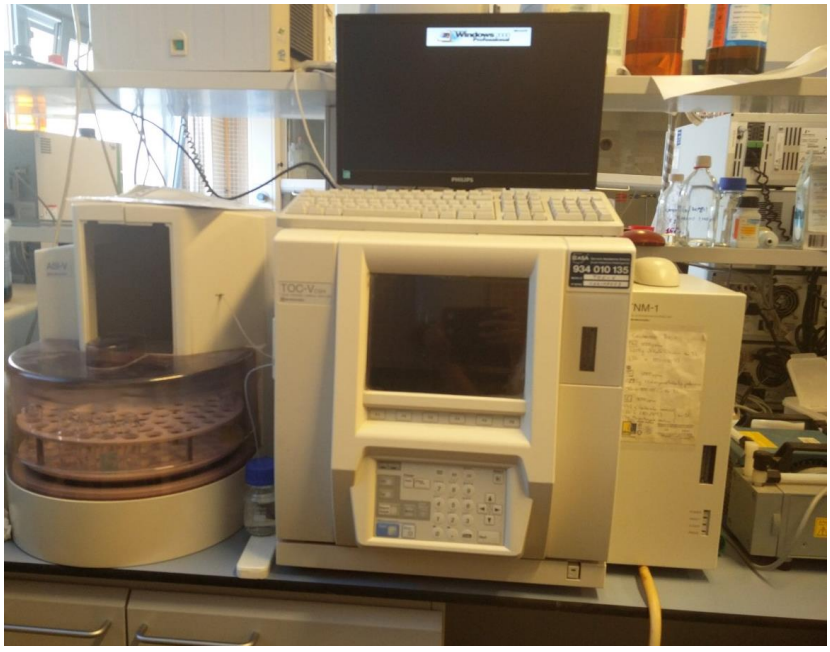


Figure 11 Principle of TOC/TN analyzer (Shimadzy Corporation. 2016)

Total organic carbon and total nitrogen were measured with Simadzu's TOC/TN analyzer which consists of the total organic carbon (TOC-V) unit, the total nitrogen-unit (TNM-1) and the automatic sample editor (ASI-v) (Picture 6).



Picture 5 TOC/TN analyzer by Shimadzu

3.5 Fluorescence spectrofluorometer

Fluorescence spectrofluorometry measures luminescence which is emitted by a matter because of the electronically excited states. Since the method is very sensitive, the luminescence can tell elaborate information about the matter on molecular level. Fluorescence spectrometry is widely used e.g in biotechnology and medical diagnostics. (Lakowicz. 2006. 1)

The principle of a simple fluorescence spectrofluorometer is presented in Figure 12. The analyzing process begins from the light source which can be e.g Xenon arc lamp or a mercury lamp. First the beam created by the lamp faces an excitation monochromator or a filter. This phase's function is to choose the desired wavelengths which are about to continue their way to the sample. The sample is usually a solution, so it is placed in a cuvette or in a flowcell. Next an emitted luminescence continues its way through emission monochromator or filter and is detected by a detector. (PerkinElmer. 2000)

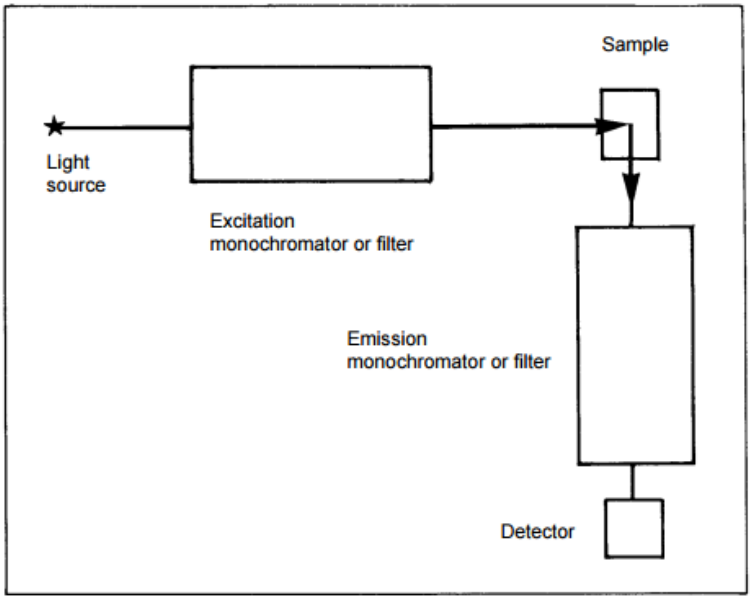


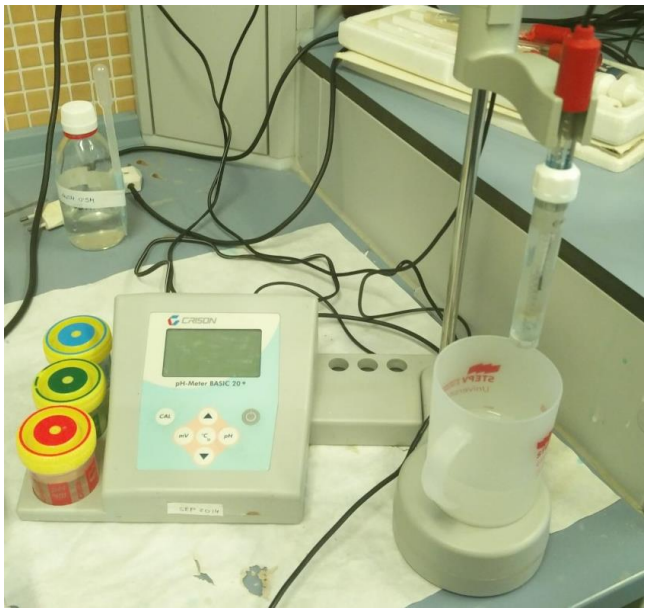
Figure 12 Principle of a simple fluorescence spectrofluorometer (PerkinElmer. 2000)

The fluorescence spectrofluorometer used in this project was QuantaMaster™ by Photon Technology International (Picture 7).

Picture 6 Fluorescence spectrofluorometer by Photon Technology International

3.6 PH-meter

PH was measured with pH-meter Basic 20 by Crison (Picture 8). Measuring happens with device's electrod which detects the pH. PH-meter has a magnetic stirrer built-in.



Picture 7 Crison Basic 20 pH-meter

3.7 Solar simulator

Photo-Fenton reaction was performed by Sun 2000 Solar Simulator by Abet Technologies (Picture 9). A Xenon short arc lamp by Ushio was used

as a source of light. The illumination of Sun 2000 Solar Simulator directs downwards. A box filled with water was used as a base of a sample during illumination.

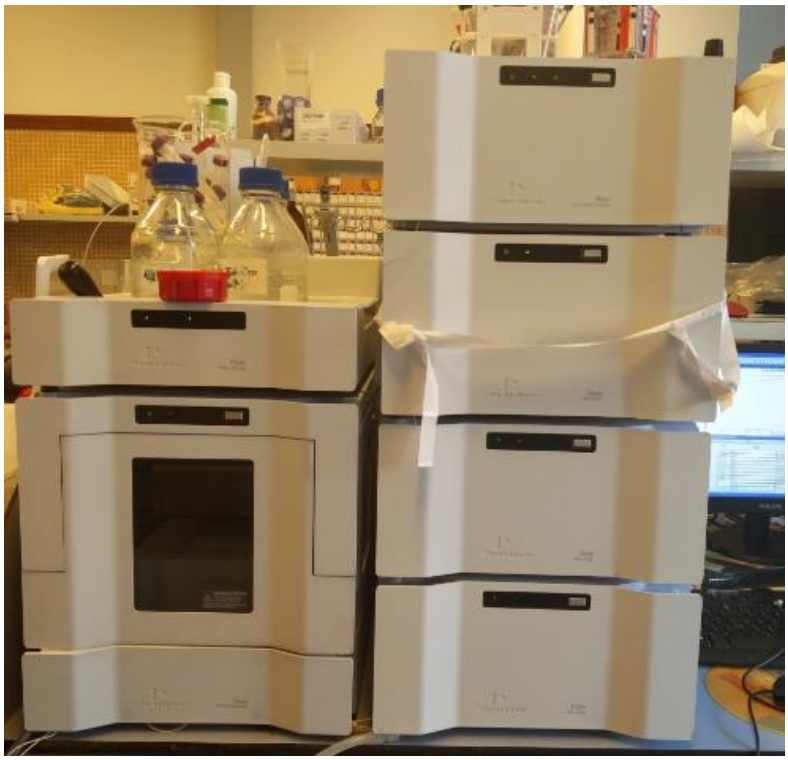
Picture 8 Sun 2000 Solar Simulator by Abet Technologies

3.8 Ultra high performance liquid chromatograph (UHPLC)

Ultra high performance chromatography (UHPLC) is a fairly new technology which was introduced in 2004. It has the same principle than HPLC, but, with a sub-2 µm column, the analyse time and the mobile phase consumption can decrease even 80% compared to the 3,5 µm column which is normally used in HPLC. Overall UHPLC offers analyses with high accuracy and short run-times. (Xu. 2013. 1)

In this project, the results of photo-Fenton reaction were analyzed with UHPLC Flexar series by PerkinElmer (Picture 10). UHPLC included the

solvent manager, UHPLC autosampler, UV/VIS UHPLC detector, the column oven and 2 UHPLC pumps.



Picture 9 UHPLC Flexar series by PerkinElmer

4 EXPERIMENTAL PART

The first sections of this chapter contain information about the samples of this project which are alperujo sample from Millena olive oil milling plant and three reference samples which were used in the characterization part of this project. Next section goes through the isolation of humic substances into an SBO-product. In the last part SBO is tested with the photo-Fenton reaction.

4.1 Sample of alperujo

The studied sample was collected from olive oil milling plant from a town called Millena, located in southern Spain. The sample was alperujo from the two-phase centrifugation process. The sample was transported to Campus of Alcoy in transparent plastic bags frozen. It was melted in room temperature before the beginning of the study and saved in the fridge of the laboratory. (Picture 11)

Picture 10 Alperujo from Millena

The percentages of humidity and organic matter were studied first by

evaporating the humidity in 105˚C and secondly burning the organic matter in 550˚C. Method is described more precisely in Figure 13.

Figure 13 The method used to examine humid-% and organic matter-%

The sample had two measurings made and their average was calculated. The results can be seen in Table 1. According to the results, alperujo from Millena was humid and rich of organic matter.

Table 1 Humidity-% and organic matter-% of alperujo

Sample	Humidity-%	Organic matter-%
$Millena_1$	59,69	97,22
$Millena_2$	59,04	97,36
Average	**59,37**	**97,28**

4.2 Reference SBO-products

In this project three different SBO-products analysis results were used as a reference in the characterization of SBO Millena. Different analysis were needed in order to know when the alperujo's composition was the most optimal to be the new SBO-product during the membrane filtration. The

characterization and comparison were done after every membrane filtration.

Three reference SBO-products were following:

- Humic acid sodium salt by Aldrich
- Pahokee peat humic acid
- Leonardite humic acid

Before analysis all the reference products were diluted with ultrapurified Milli-Q water to obtain the concentration 1:20.

According to the measurings made with spectrophotometer, all the UV-Vis spectras decrease exponentially when the lighwave is increased. Leonarditee humic acid and Pahokee humic acid have a high peak at 200 nm whereas Aldrich's humic acid sodium salt's peak stays under absorbance 1,5. (Figure 14-16)

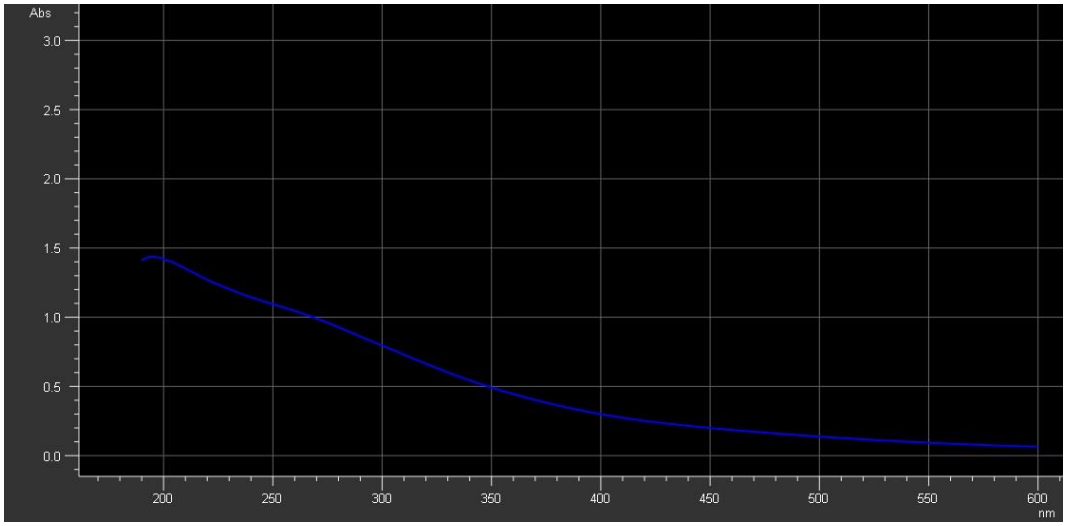


Figure 14 Uv-Vis spectra for humic acid sodium salt by Aldrich

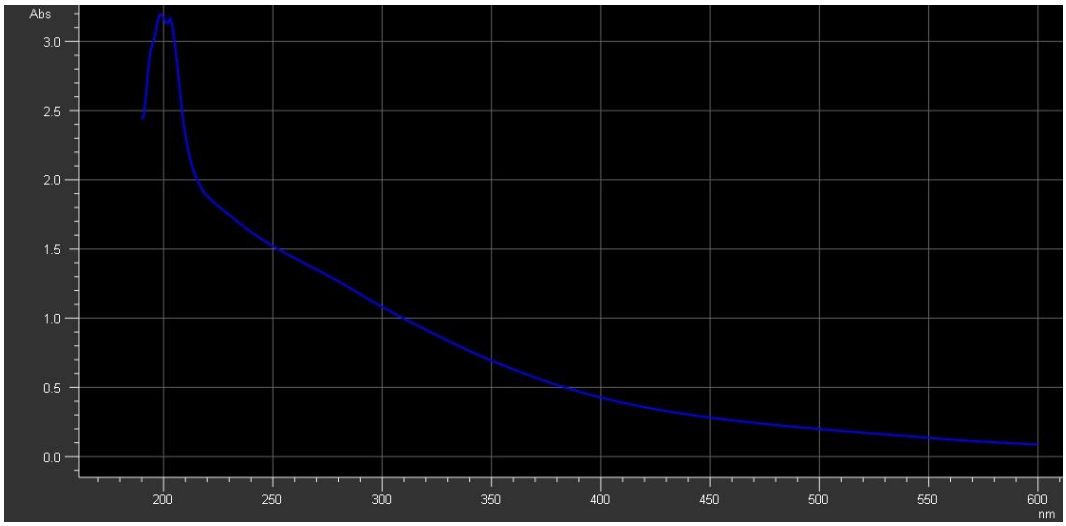


Figure 15 Uv-Vis spectra for Pahokee peat humic acid

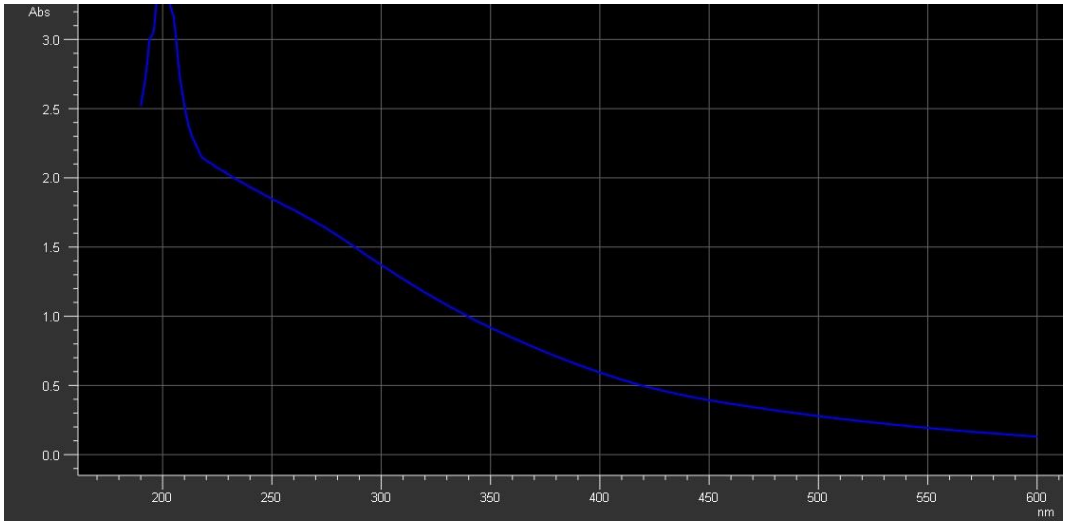


Figure 16 Uv-Vis spectra for Leonardite humic acid

Fluorescenes of the reference products were measured with spectrofluorometer and the results are presented in Figures 17-19. They all have an excitation peak at ex 450-470, em 500-550 and a very strong peak at ex 275-300, em 450-600. These peaks are considered to originate

from humic substances. The shapes of the fluorescences are also similar having the highest points about at ex 500-550.

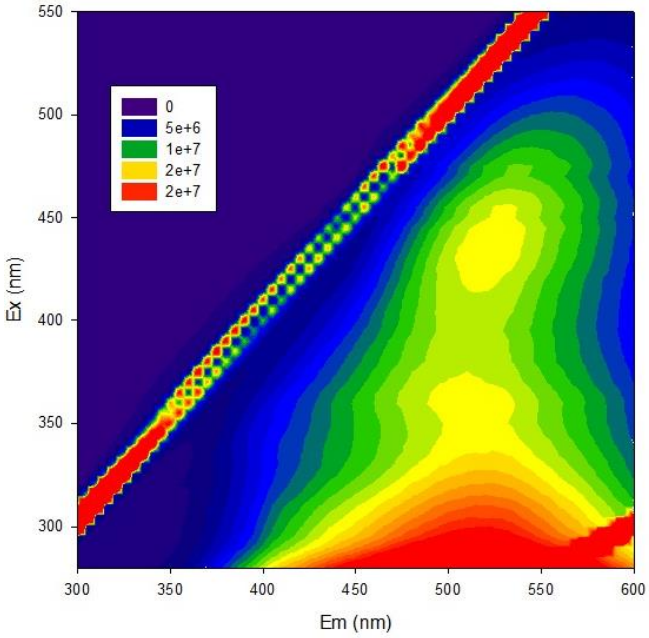


Figure 17 Fluorescence for humic acid sodium salt by Aldrich

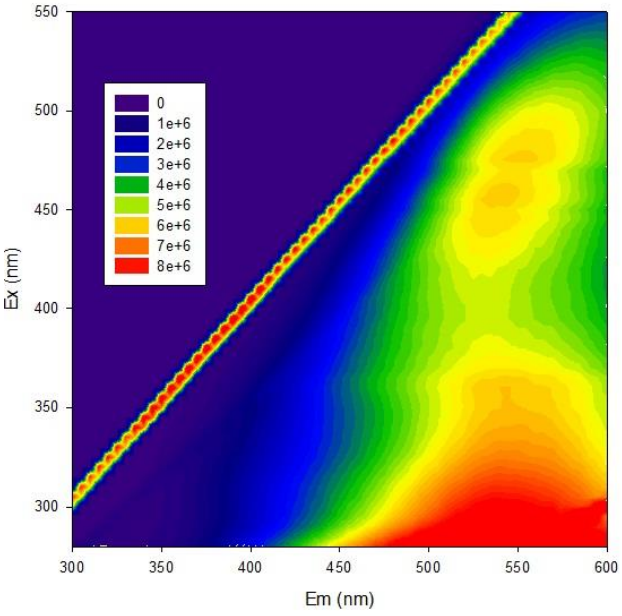


Figure 18 Fluorescence for Pahokee peat humic acid

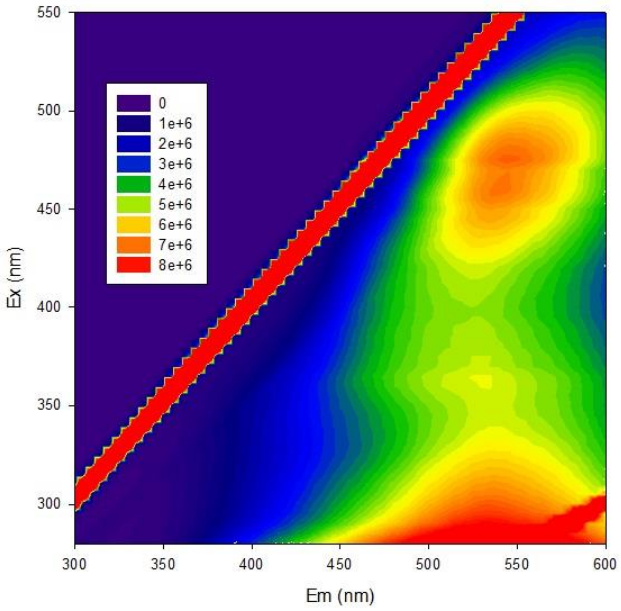


Figure 19 Fluorescence for Leonardite humic acid

Retention times and intensitys of the reference products were analysed by the liquid chromatograph. The results are presented as graphs (Figure 20) and numbers (Table 2). Area calculated from retention time and intensity can be seen in Table 2. Area is equal to the concentration of the substance at the certain retention time. According to the results, humic acid sodium salt by Aldrich and Leonardite humic acid have the same retention time 12,56 min whereas Pahokee humic acid has a bit shorter time 12,31 min. After these results the retention time for humic substances can be assumed to be around 12-13 min. Humic acid sodium salt and Leonardite humic acid seem to have quite similar concentration of humic acids with areas 209505 and 405499, where as Pahokee peat humic acid has a bigger concentration with area 1093422.

Table 2 Liquid chromatgraph results of the reference products

Sample	Retention time	Area
Humic acid sodium salt by Aldrich	12,56	309505
Pahokee humic acid	12,31	1093422
Leonardite humic acid	12,56	405499

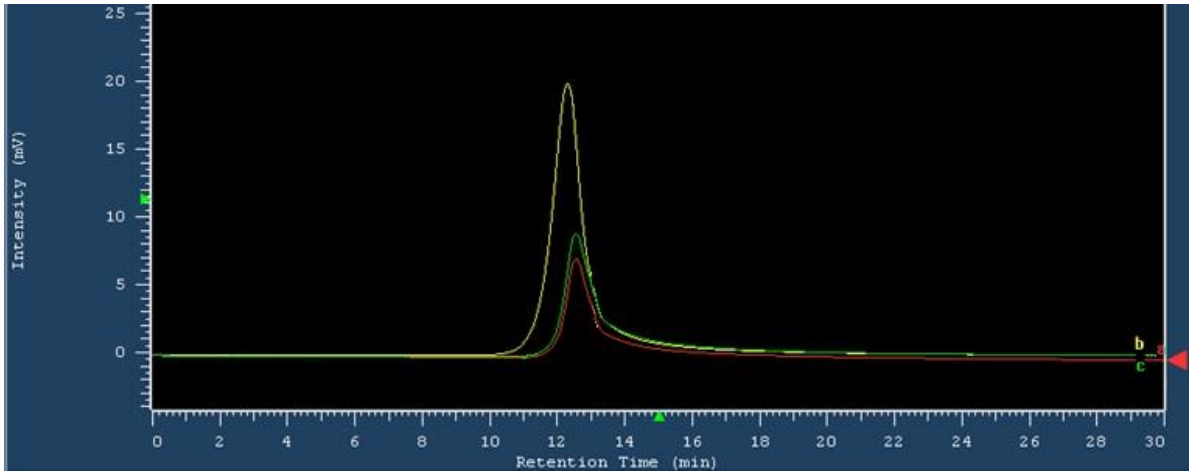


Figure 20 Liquid chromatograph results where Red = humic acid sodium salt by Aldrich, Yellow = Pahokee peat humic acid, Green = Leonardite humic acid

Total organic carbon and nitrogen of Leonardite and Pahokee peat humic acids were measured by TOC/TN analyzer (Table 3). The results were quite similar for both products: TOC was around 24,5 mg/l, TC 57-58 mg/l and IC 32-33 mg/l. The biggest difference can be seen in the amount of TN, but both products contain nitrogen less than 2 mg/l.

Table 3 TOC/TN results for Pahokee peat humic acid and Leonardite humic acid

Sample	Total organic carbon (TOC)	Total carbon (TC)	Inorganic carbon (IC)	Total nitrogen (TN)
Pahokee peat humic acid	24,35	56,98	32,63	1,97
Leonardite humic acid	24,91	57,79	32,88	0,76

4.3 Isolating humic-like substances from alperujo

This chapter describes how the isolation process was performed. The process consisted of the isolation with different filters and the characterization of each sample afterwards to tell if the constitution of the sample was close to the commercial SBOs.

4.4 Digestion

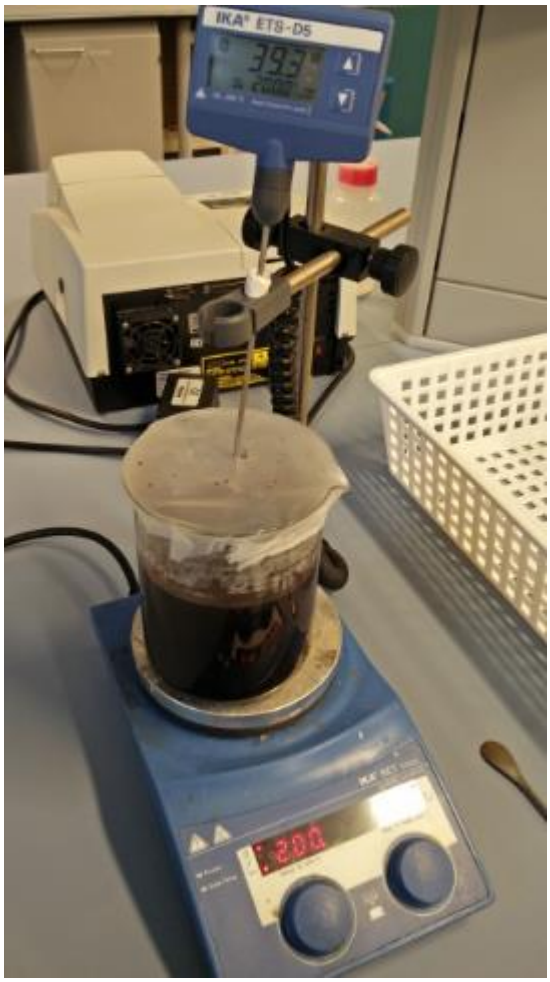
The molecular weight of humic substances is known to vary from 67 to 463 kg mol-1 (Gomis et al. 2014. 177). Digestion method was used to break the molecules to their prime factors. After the digestion it was possible to isolate the molecules of humic substances.

Digestion method was the same that was used in Italy in a project where UBW was turned into SBO (Montoneri et al. 2011). Accoding to the known method the sample was mixed with potassium hydroxide (KOH) and Milli-Q water to generate an alkali solution of pH 13. The volume of the solution was 0,5 l and the molarity of KOH 0,3mol. According to the molecular weight of KOH which is 56,11g/mol, 8,4g of KOH was needed to obtain the right molarity to the solution (Equation 4 & 5).

$$M = \frac{n}{V(l)} \rightarrow n = MxV(l) = 0{,}3Mx0{,}5l = 0{,}15mol \quad (4)$$

$$n = \frac{m}{M_r} \rightarrow m = nxM_r = 0{,}15mol\ x\ 56{,}11\ g/mol = \ 8{,}4g \quad (5)$$

Digestion was performed by a magnetic mixer with a stove and a temperature-meter (Picture 12). The solution was heated up until the temperature was 65 ºC and the digestion was kept going on for 4 hours. The pH of the solution was measured with a pH-meter before and after the digestion to ensure it was 13.

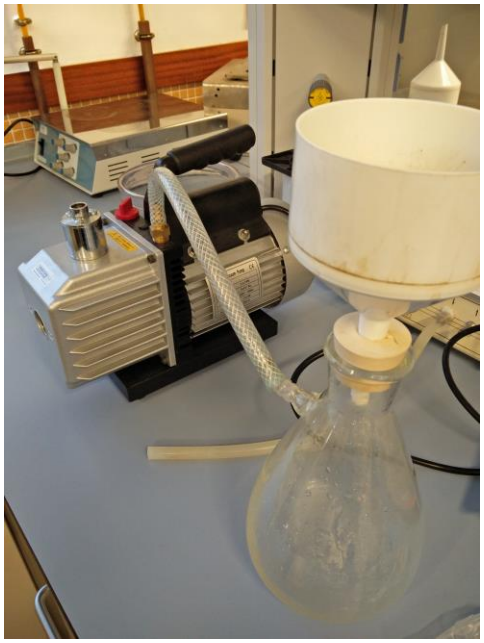


Picture 11 Digestion equipment

4.5 Fabric filtration

The digested solution was first filtered through fabric filters in order to separate the biggest particles of soil from the solution. In this process a 2-staged vacuum pump was used to enhance the filtration (Picture 13). After

the fabric filtrate was so full of soil particles that it didn’t let any liquid pass through anymore it was cleaned and the soil was moved to an another glass. After the solution was filtrated the leftover soil particles were washed with 0,3 M KOH-solution of pH 13 until the KOH-solution mixed up with soil particles was transparent. This phase was done to ensure that all the humic substances were recovered. Next KOH-solution was added to the filtered solution until there was 5 litres of liquid obtained (Picture 14). This was done because membrane filtration process of this work required 5 litres of solution to function properly.



Picture 12 Filtration equipment

Picture 13 Sample solution after fabric filtration

4.6 Membrane filtration

After the solution was filtered with fabric filters it needed to be filtered with membranes to obtain the specific molecule size of humic substances. The sample solution was characterized after every filtration, which was done overall three times until the final constitution. Membrane filtration divided the sample solution into 2 parts: Into the filtrated (F) part which had the molecules smaller than the membrane holes and into the retained (R) part which had the molecules which could not go through the holes of the membrane. The size of the molecules could be seen as clarity in the sample solutions (Picture 15). The aim was to get as much of humic substances as possible into either of F or R solutions.

Picture 14 F and R after membrane filtration with membrane size 0,14m.

First filtration was performed with micro membrane size 0,14 µm and the second and third one with ultra membrane sizes 150 kg/mol and 50 kg/mol.

4.7 Characterization of the sample

R and F solutions were characterized to find out if the composition of either of them was close to the commercial SBO-products. Characterization was done after every membrane filtration. Solutions were diluted for analysis with Milli-Q-water.

4.7.1 Characterization of the sample filtered with membrane size 0,14 µm

The first membrane filtration was performed with micro membrane size 0,14 µm. Because this was the first characterization, overall six dilutions were made to have a general view of the sample. Following dilutions were made with Milli-Q water:

- R 1:200
- R 1:100
- R 1:20
- R 1:2
- F 1:20

- F 1:50

Analysis with spectrophotometer were done to find out which solutions were suitable for spectrofluorometer analysis. Analysis results got with spectrofluorometer can become distorted if the sample absorbs so much light in certain wavelengths that it turns into a false emission at the same time. The samples having absorbation less than 1,5 in wavelength 250 nm were taken to the spectrofluorometer analysis. The spectrophotometer results for R solutions are presented in Figures 21-23. According to the results, R 1:200 and R 1:100 were suitable to be analyzed with spectrofluorometer.

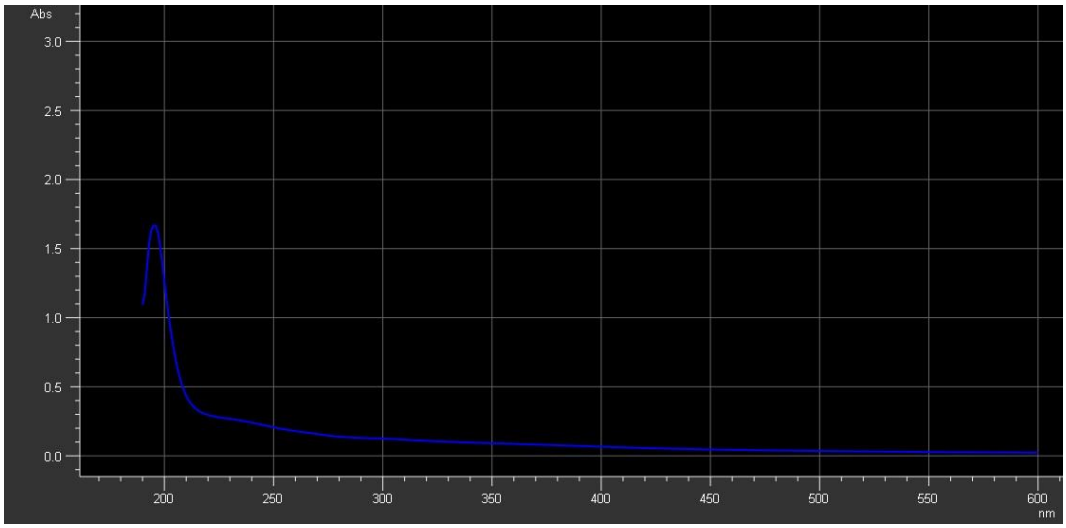


Figure 21 Uv-Vis spectra for 14µm R 1:200

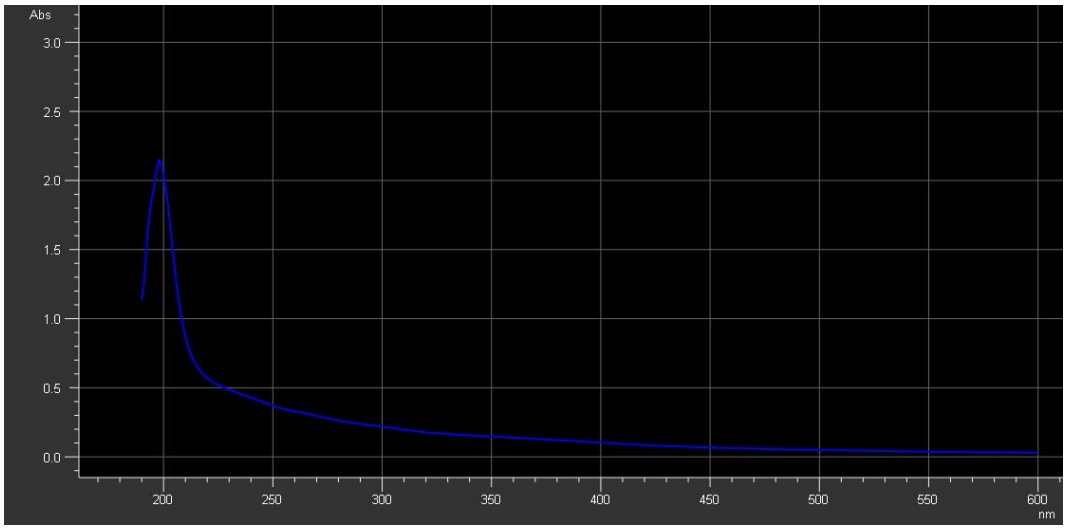


Figure 22 Uv-Vis spectra for 14µm R 1:100

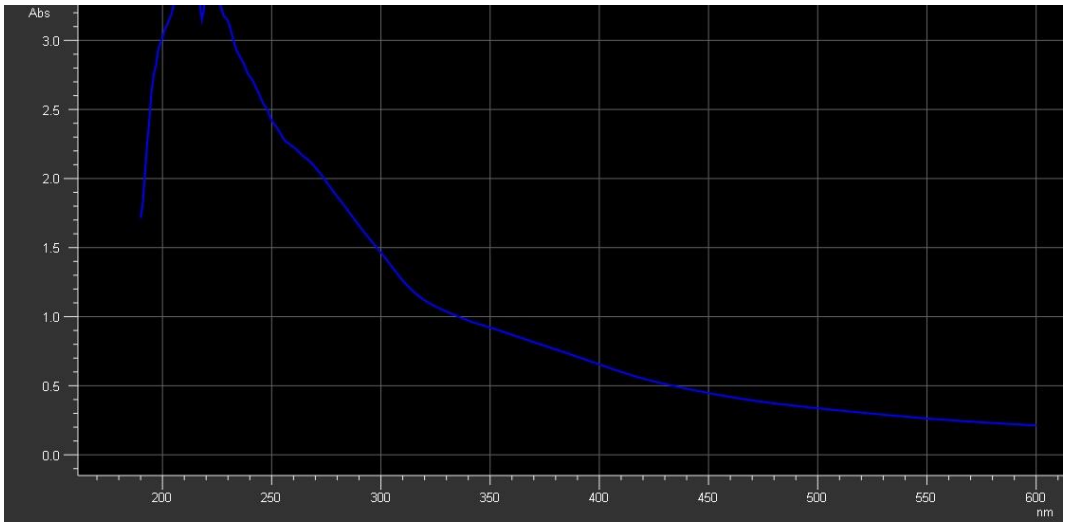


Figure 23 Uv-Vis spectra for 14µm R 1:20

Spectrophotometer results of the filtrated solutions can be seen in Figures 24 and 25. According to the results both of them were suitable for spectrofluorometer analysis.

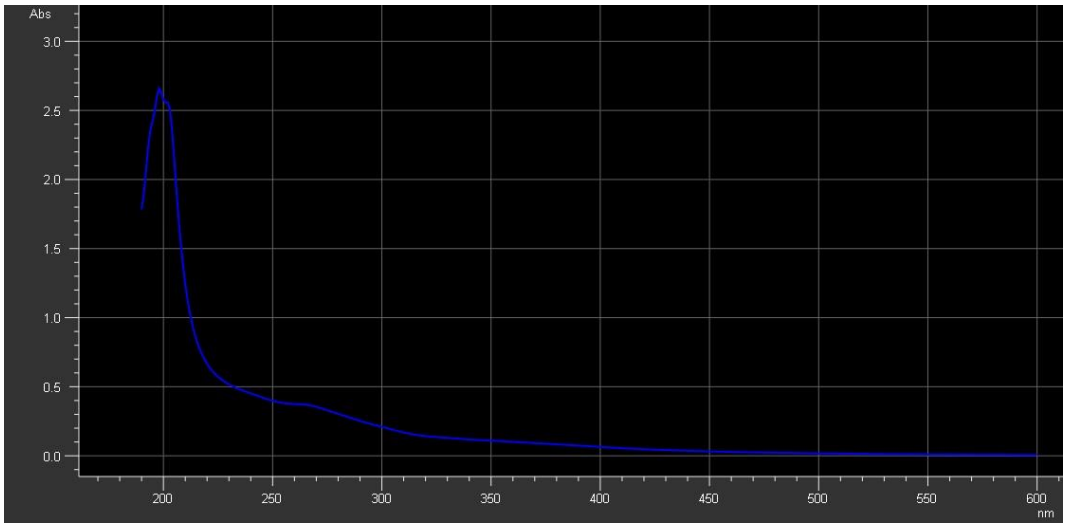


Figure 24 Uv-Vis spectra for 14µm F 1:50

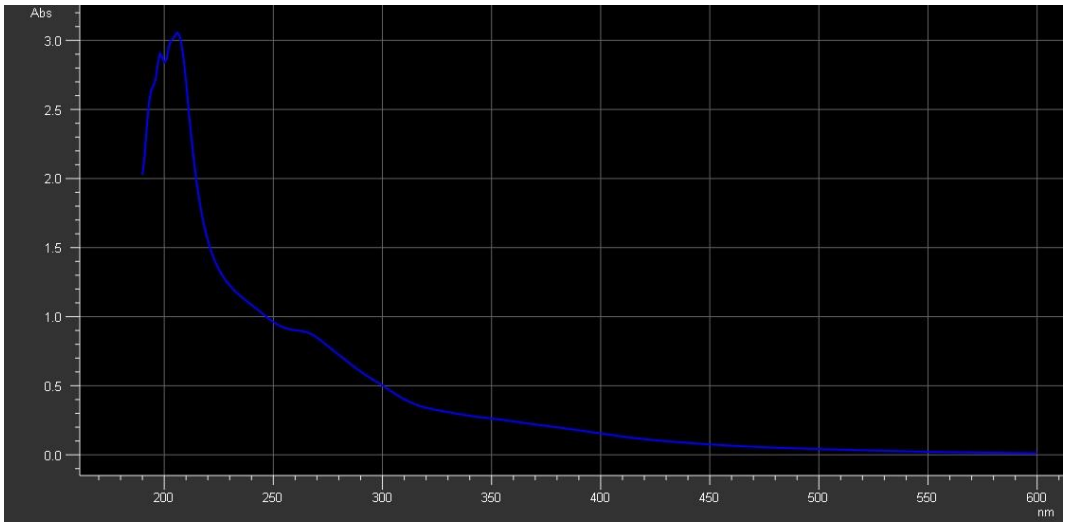


Figure 25 Uv-Vis spectra for 14µm F 1:20

Fluorescenes for R 1:100, R 1:200, F 1:20 and F 1:50 are presented in Figure 26 and 27. The fluorescenes of the filtrated solutions show a shape close to the commercial products.

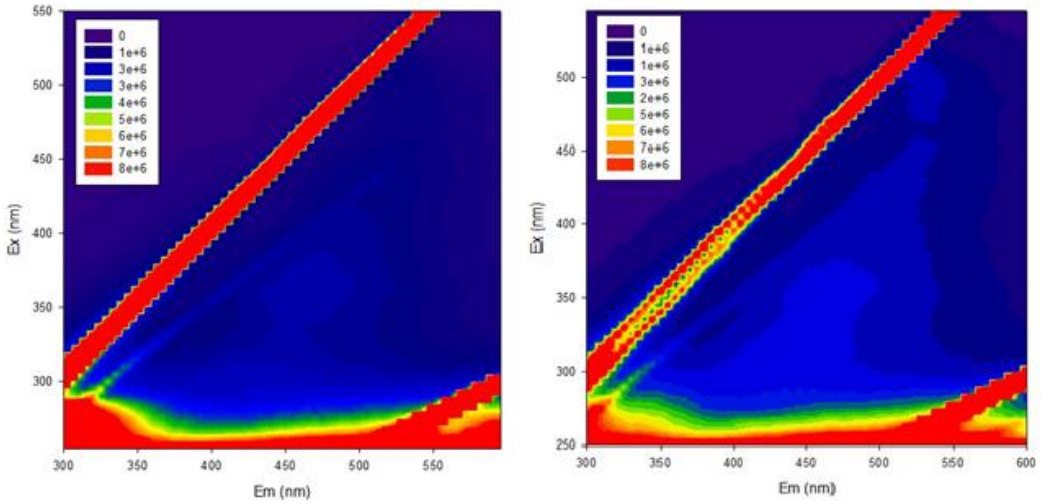


Figure 26 Fluorescenes for R 1:200 (left) and R 1:100 (right)

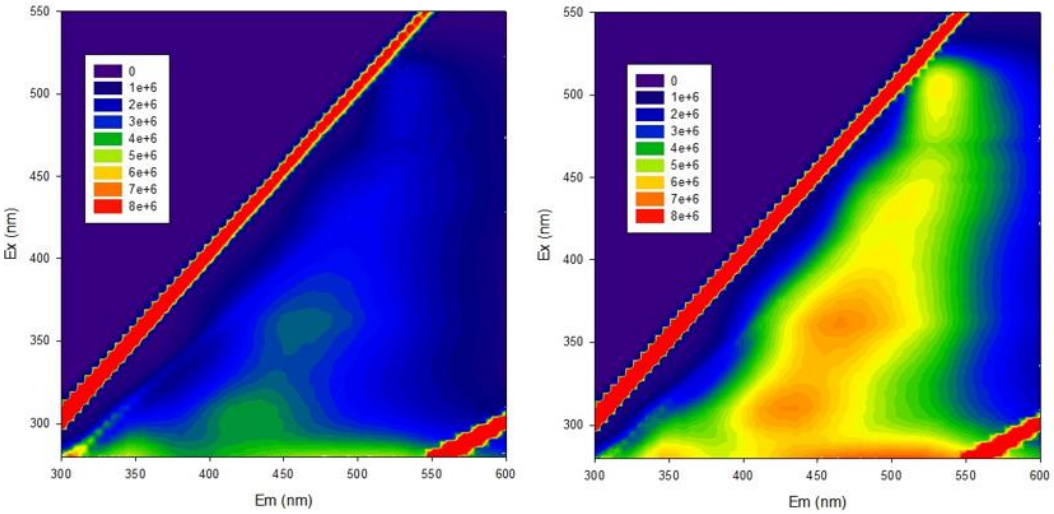


Figure 27 Fluorescenes for F 1:50 (left) and F 1:20 (right)

Liquid chromatograph analysis were performed with 100% tampon phosphate as an evaluation liquid. According to the analysis results of the commercial SBO-products there should be a peak in retention time 12,513 with area somewhat 215332.

Results for solutions R 1:100, F 1:20 and F 1:50 are presented as graphs in Figure 28 and numbers in Table 4. Every graph has a peak in retention time 12-13 min, so the sample could be assumed to contain humic

substances at this point. Another peak can be seen in retention time ~16 min which means there is also something else besides humic substances.

Table 4 Liquid chromatograph results for R 1:100, F 1:20 and F 1:50

Sample	Retention time	Area
R 1:100	12,71	283261
	16,29	362821
F 1:20	12,72	1203266
	16,20	364879
F 1:50	12,78	364879

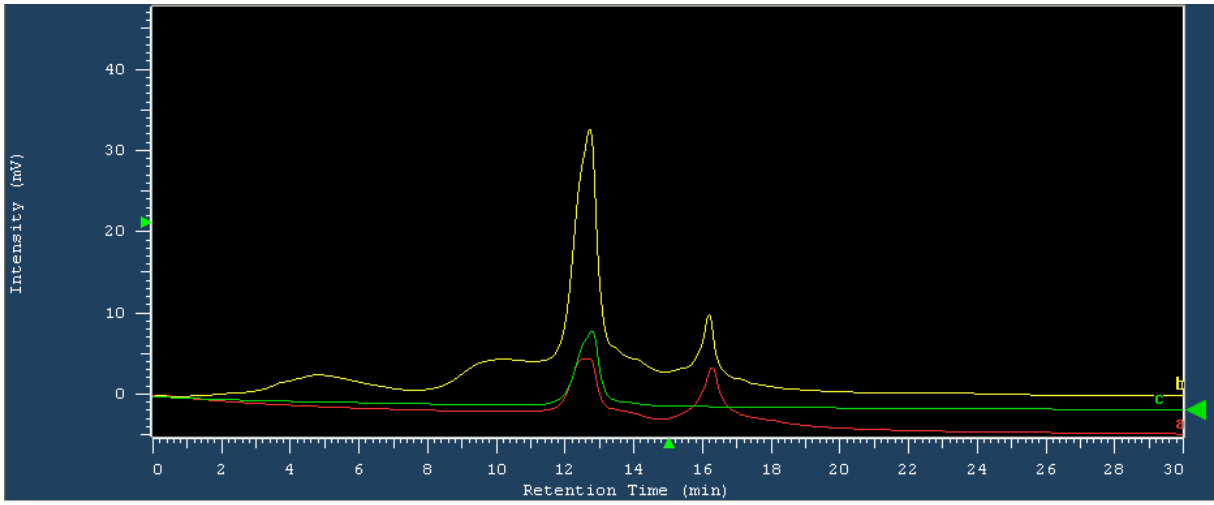


Figure 28 Yellow: F 1:20, Green: F 1:50, Red: R 1:100

After the first filtration it was confirmed that the sample contained humic substances. The filtration was continued with a smaller membrane size to get closer to the optimal composition close to the commercial SBO-products.

4.7.2 Characterization of the sample filtered with membrane size 150kg/mol

First R and F were diluted to obtain following dilutions:

- R 1:20 retentato
- F 1:20 filtrado
- F (no dilution)

Spectrophotometer results are presented in Figures 29-31. According to the results R 1:20 and F 1:20 were suitable to be measured with the spectrofluorometer.

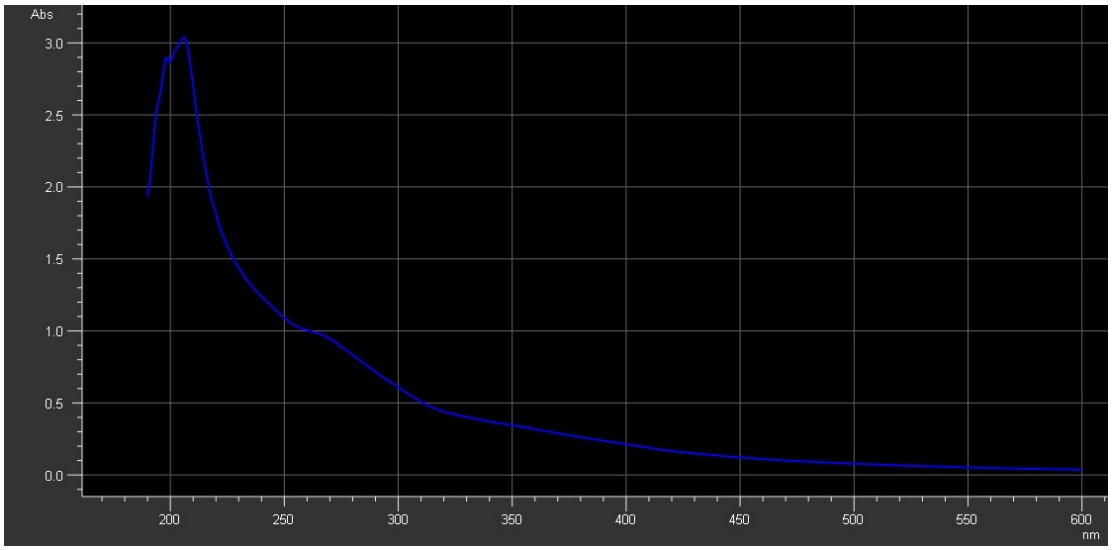


Figure 29 Uv-Vis spectra for 150kg/mol R 1:20

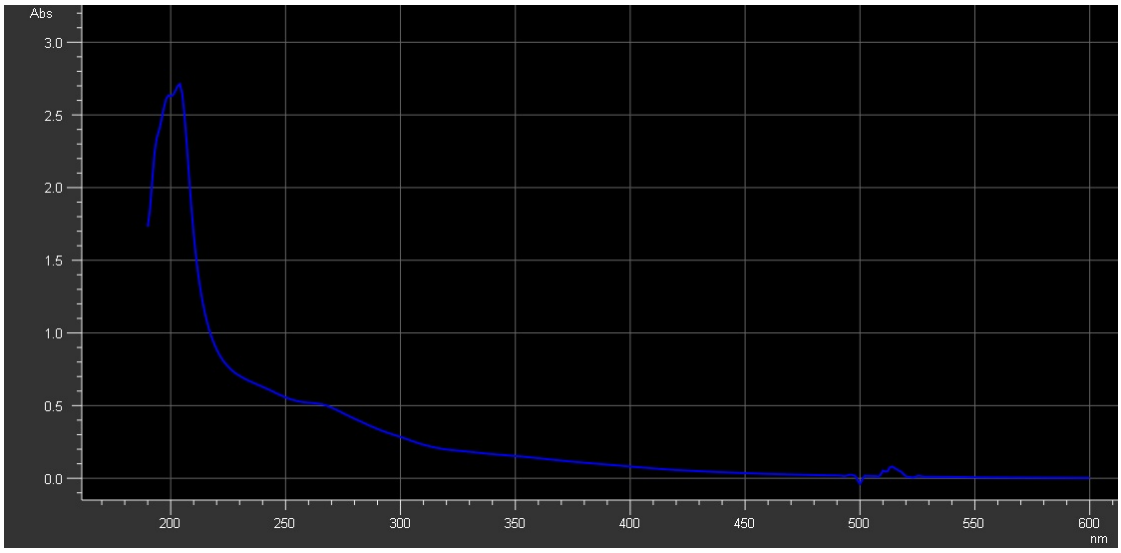


Figure 30 Uv-Vis spectra for 150kg/mol F 1:20

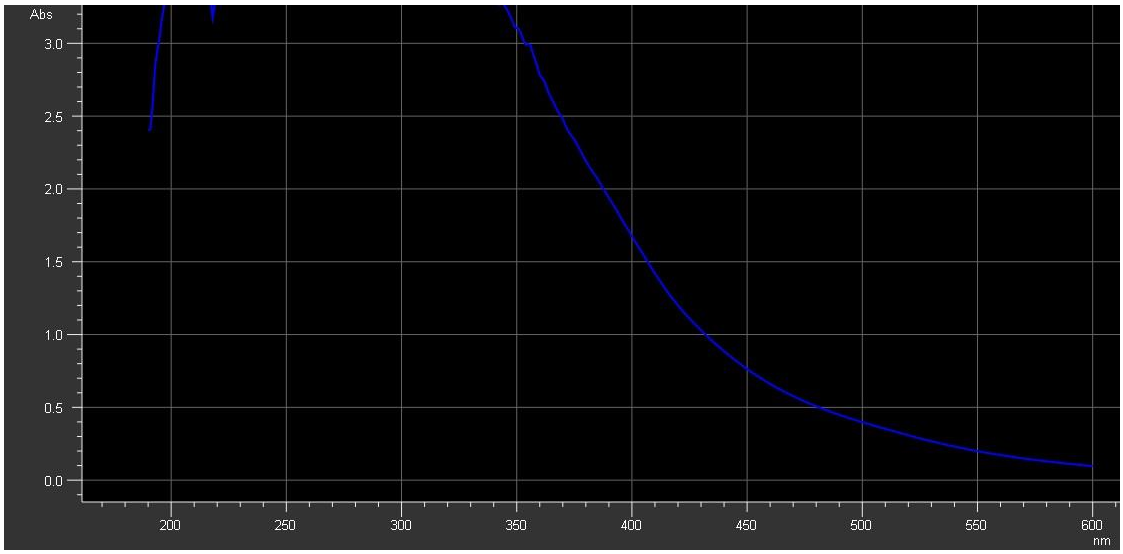


Figure 31 Uv-Vis spectra for 150kg/mol F 1:1

Fluorescences of R: 1:20 and F 1:20 are presented in Figure 32. Both of the fluorescences had strong excitation at em 300-600, ex 200-230 and peaks at em 425, ex 300 and em 450, ex 350. In F solution the peaks seemed to be clear, whereas R solution had excitation in wider area.

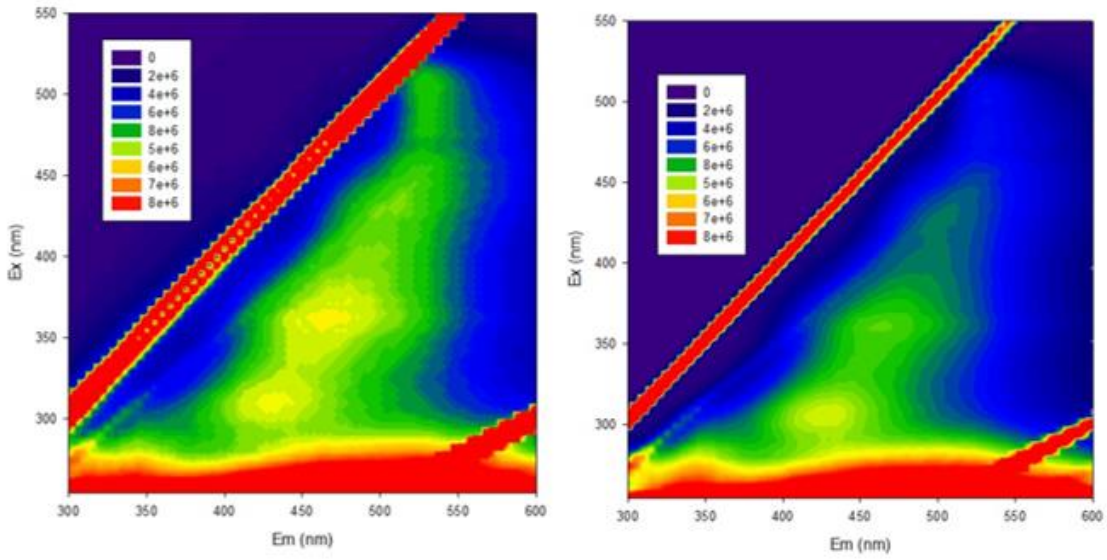


Figure 32 Fluorescenes for R 1:20 (left) and F 1:20 (right)

The liquid chromatograph results for solutions R 1:100, F 1:20 and F 1:50 are presented as numbers in Table 5 and as graphs in Figure 33. Now every graph contained only one peak in retention time 12-13 min. Diluted R and F had quite similar retention times and areas meaning that humic substances could still be found from both solutions.

Table 5 Liquid chromatograph results for R 1:20, F 1:20 and F

Sample	Retention time	Area
R 1:20	12,55	868241
F 1:20	12,78	581370
F	12,78	15740518

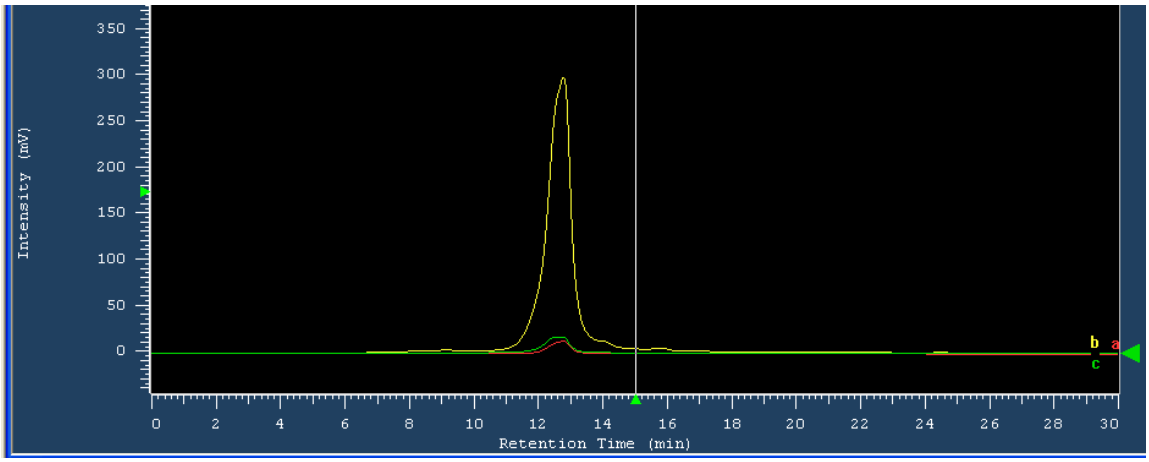


Figure 33 Green: R 1:20, Red: F 1:20, Yellow: F

After the results of 150kg/mol filtration, the sample needed to be filtrated with smaller membrane size because it seemed that humic substances were still divided quite evenly into the both solutions.

4.7.3 Characterization of the sample filtered with membrane size 50kg/mol

This was the final membrane filtration. Following dilutions were prepared for analysis:

- F without dilution
- F 1:20
- F 1:10
- F 1:2
- R without dilution
- R 1:20

Spectrophotometer results for F 1:20, F 1:10, F 1:2 and R 1:20 are presented in Figures 34-37. According to the results R 1:20 and F 1:10 were suitable to be measured with the spectrofluorometer. Normally F 1:10 absorbation would had been too high for the spectrofluorometer but an

exception was made this time because the solution was rather clear at this point.

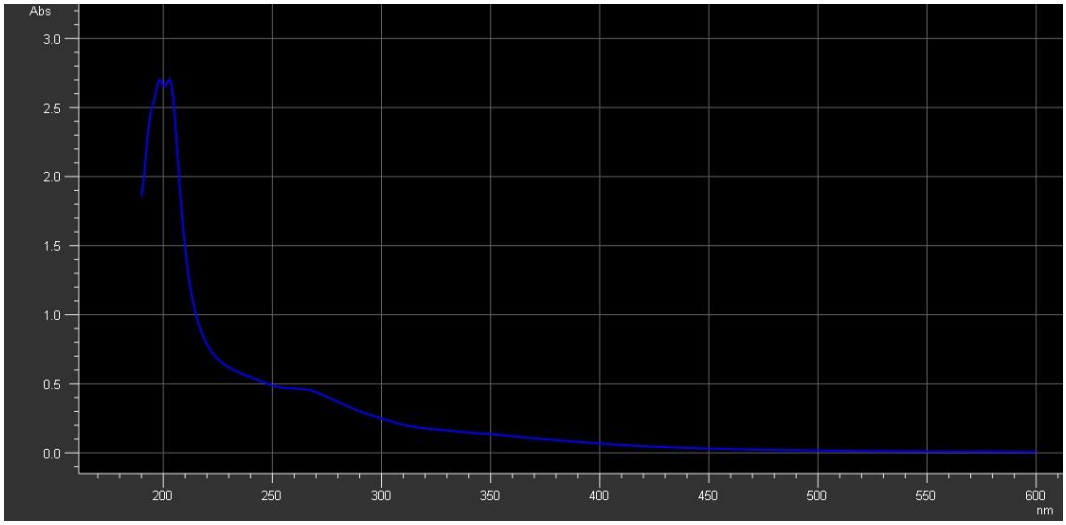


Figure 34 Uv-Vis spectra for F 1:20

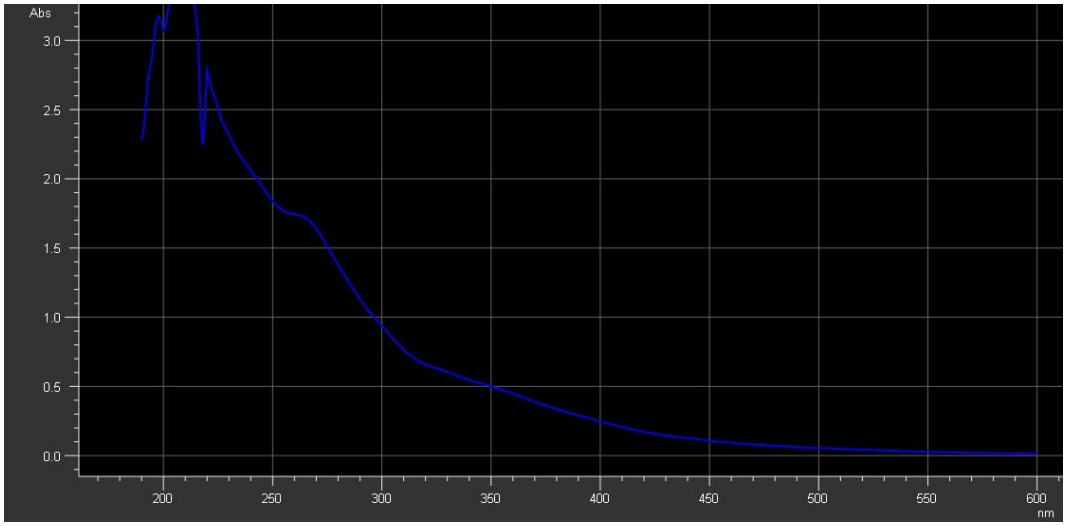


Figure 35 Uv-Vis spectra for F 1:10

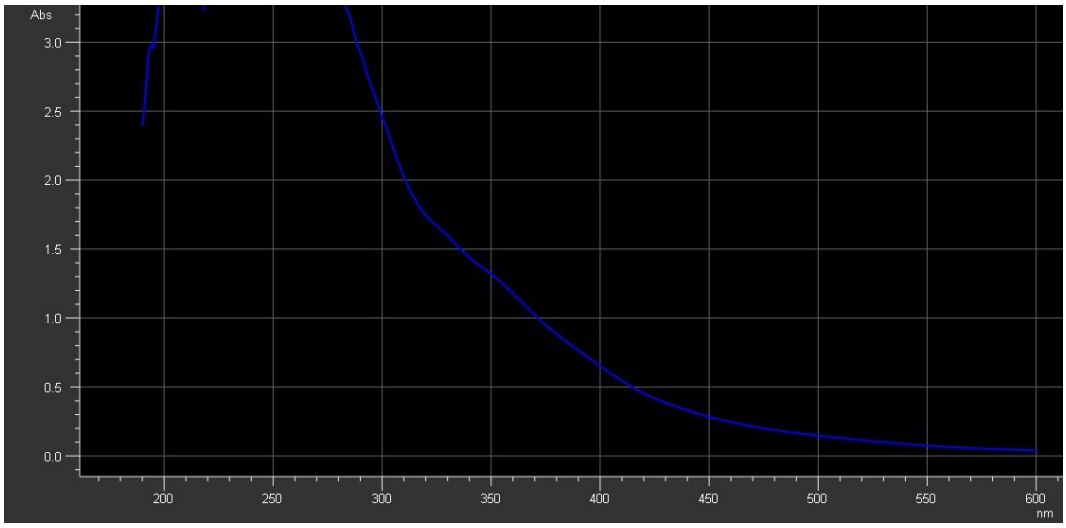


Figure 36 Uv-Vis spectra for F 1:2

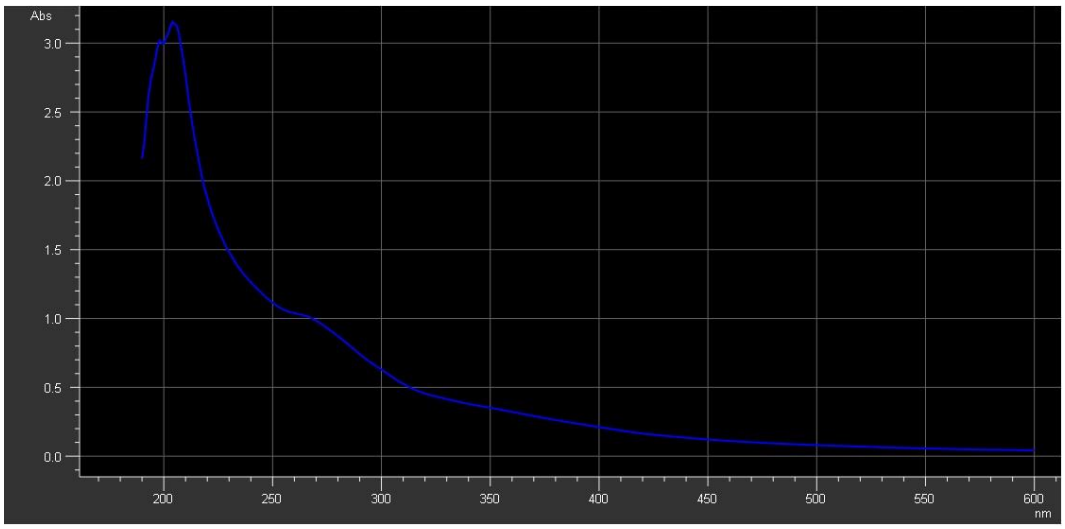


Figure 37 Uv-Vis spectra for R 1:20

Fluorescenes for R 1:20 and F 1:10 are presented in Figure 38. The both fluorescences have similar shapes but in F's fluorescence excitation is strong at wide area whereas in R's fluorescence shows peaks at em 450-550, ex 230, em 440, ex 310 and em 360, ex 370.

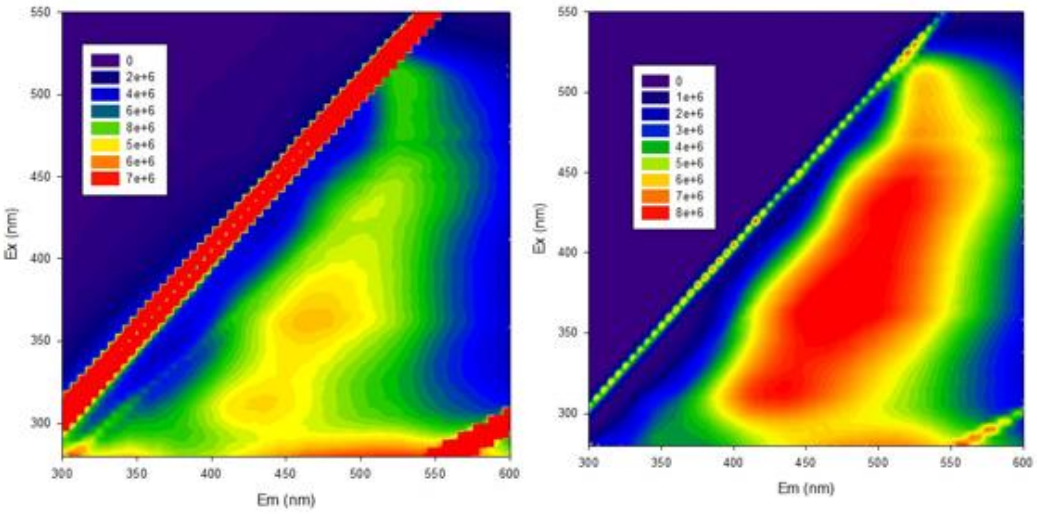


Figure 38 Fluorescenes for R 1:20 (left) and F 1:10 (right)

Liquid chromatograph results for solutions R without dilution, R 1:20, F without dilution and F 1:20 are presented as numbers in Table 6 and as graphs in Figure 39. The graphs show that retention time of F without dilution is slightly over 13 min whereas R has retention time 12,58 min with larger area than F.

Table 6 Liquid chromatograph results for R, R 1:20, F and F 1:20

Sample	Retention time	Area
R	12,58	21946365
R 1:20	12,62	882712
F	13,02	13158595
F 1:20	12,73	441622

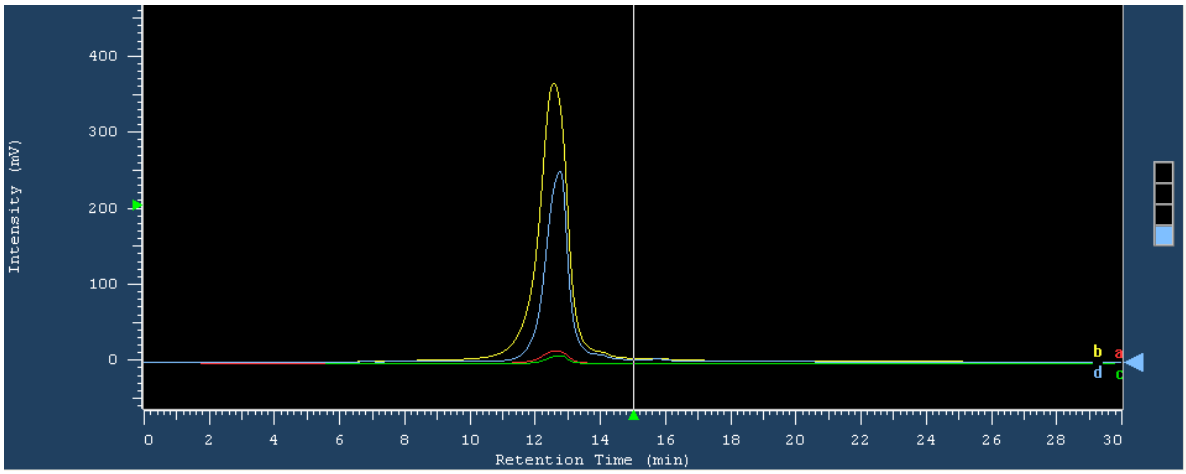


Figure 39 Yellow: R 1:1, Blue: F 1:1, Red: R 1:20, Green: F 1:20

According to there results R solution after filtrating membrane size 50 kg/mol was chosen to be the SBO-product of this project. The UV-Vis spectra of R was very similar with the spectras of the commercial SBO-products, fluorescence had clear peaks at the points assumed to be originated from humic-like substances and the graph obtained by liquid chromatograph showed a typical retention time for humic-like substances with a large area.

4.8 Turning the solution into solid form

The solution was turned into solid form with an oven treatment. The solution was put in the oven for 24 h with temperature 60 ºC. After the treatment liquid was evaporated and solid particles, including humic-like substances, crystallized forming a solid SBO-product (Picture 19). Millena SBO's characterization can be seen in the results part of this study.

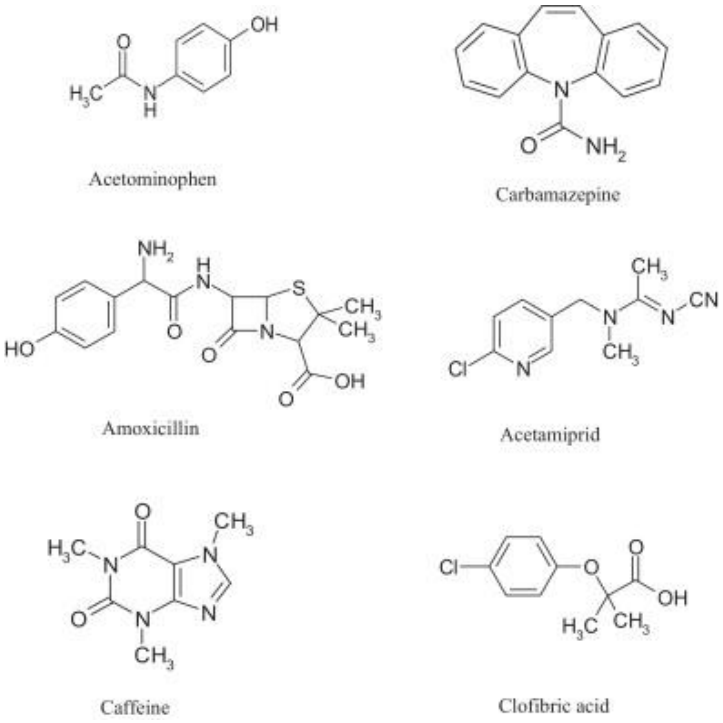
Picture 15 Final SBO-product

4.9 Performing photo-Fenton reaction with SBO Millena

SBO Millena's function as a chemical auxiliary was tested by photo-Fenton reaction. Photo-Fenton reaction is one of Fenton-reactions which are effective methods for waste water treatment. Reactions require acidic pH to keep the iron ions in the reaction. With SBO, it is possible to perform the reaction with neutral pH. Here the reaction is performed with pH 5. This project's reaction circumstances and method were adapting the known method which was done in Campus of Alcoy with SBO from Italy (Gomis et al. 2014).

4.10 Reagents

A solution of 6 different pollutants was prepared for the reaction. The contaminants were acetaminophen, acetamiprid, amoxicillin, caffeine, clorifibic acid and carbamazepine (picture 20). Concentration of each contaminant was 5 mg/l diluted with Milli-Q water.



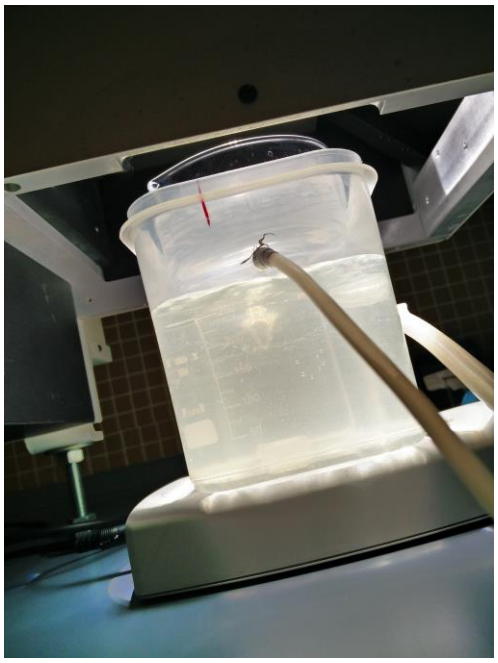
Picture 16 Chemical structures of the contaminants (J. Gomis et al. 2014. 40)

The reaction was done with iron (Fe), hydrogen peroxide (H_2O_2) and SBO. Concentration for Fe was 4 mg/l, for H_2O_2 2,2 mmol and for SBO 20 mg/l.

4.11 Reaction

First the contaminant solution was poured in a 250 ml beaker with 4,84 mg Fe and 5 mg SBO. Then pH was measured with pH-meter. If it was higher or lower than 5, it was modified by increasing it by adding one drop of 0.1 M sodium hydroxide (NaOH) or decreasing it by adding one drop of 0.1 M sulfuric acid (H_2SO^4). The final pH of the reaction was 5,18. The solution with neutral pH was put in the solar simulator under a radiation source which was 550 W Xenon arc lamp (picture 18). A Pyrex gass filter was used to exclude the certain wavelengths. The reaction was started by adding 57,5 µl of H_2O_2 to the solution. At the same time a timer was started. Samples were taken at the times (min) 0, 2, 5, 7, 10, 15, 20, 30,

40, 60 and 90. First zero was taken when the solution contained only the contaminants, Fe and SBO. This zero can be seen in results at t=-10.



Picture 17 Photo-Fenton reaction

Before taking a sample from the beaker the timer was stopped and the solar simulator's shutter put on. Approximately 2 ml of sample was taken with a pipette, after that the shutter was turned back off and the timer on immediately to get the most objective results as possible. Samples were filtered through a 4,5 µm polypropylene membrane to a glass. 1,5 ml of filtered sample was taken with a pipette, and added in a 2 ml vial with 0,3 ml of methanol . Because of methanol, the reaction did not continue in a vial and the results did not become distorted.

The photo-Fenton reaction was performed without SBO as well to get results for comparison. The solution without Millena SBO had pH under 4 so it was modified to 5,32 with NaOH. After the reaction, pH was decreased back to 3,7 so the reaction without SBO could not be done under neutral media completely.

The samples were analyzed with UHPLC by PerkinElmer. The results are presented in the results part of this study.

5 RESULTS

The results part is divided into 2 parts. The first part goes through the characterization of the Millena SBO and the second part presents the results of the photo-Fenton reaction.

5.1 Characterization of Millena SBO

Characterization of the final product was performed in the same way as the earlier ones. Solid Millena SBO was diluted with Milli-Q water to obtain a dilution 1:20.

The spectrophotometer result for Millena SBO is presented in Figure 40. The highest peak is at absorbance 3,0, wavelenght 200 nm. Absorbance decreases quite exponentially when wavelength is increasing. At 250-270 nm absorbance stays in 1.0 but continues decreasing after 270 nm.

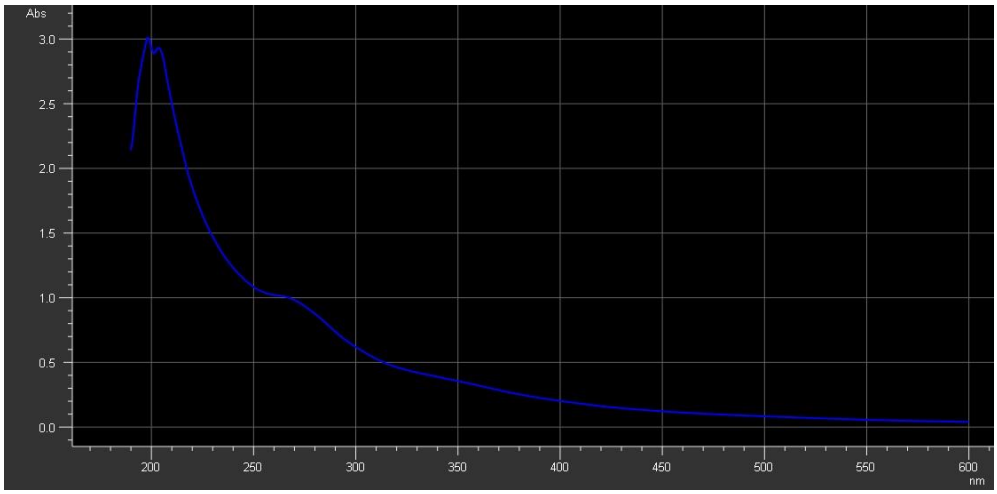


Figure 40 Uv-Vis spectra for final SBO 1:20

Comparison with the spectras of the commercial products show a high similarity with Pahokee humic acid (Figure 41). Both of them have a peak in 200 nm close to 3,0 absorbance. The graph of Millena SBO is also quite similar with Leonardite humic acid. Millena SBO's absorbance decreases a

bit more at 200-250 nm than Pahokees and Leonardites. One common factor, which can be seen in every spectra, is the absorbance increasing exponentially when wavelength is decreasing. Also the highest absorbance point of every spectra is approximately at 200 nm.

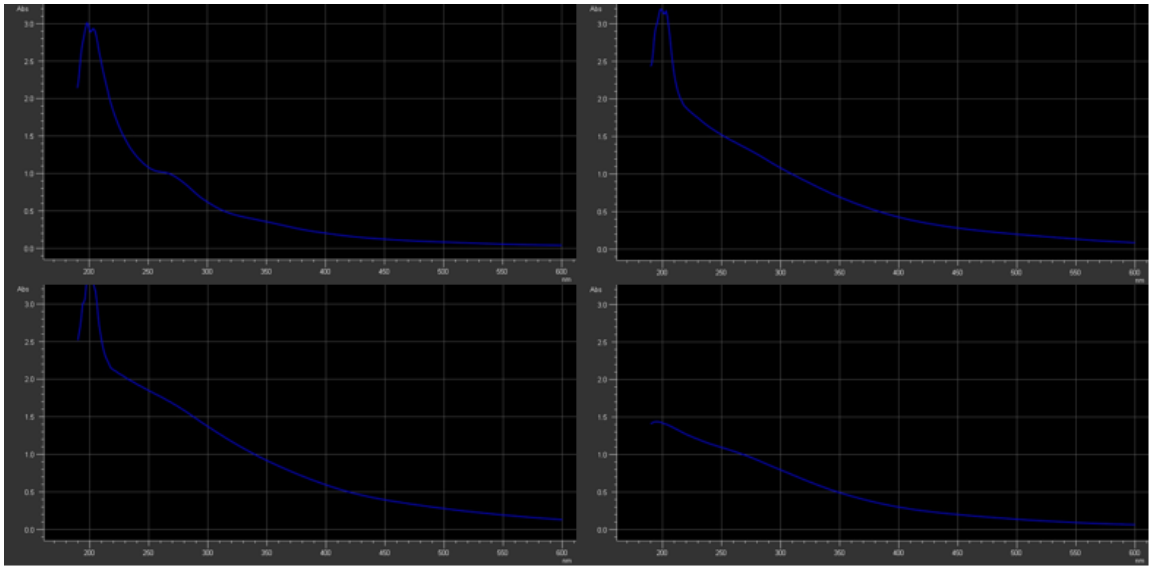


Figure 41 Uv-Vis spectras for Millena SBO (upper left), Pahokee humic acid (upper right), Leonardite humic acid (down left) and humic sodium salt by Aldrich (down right)

Fluorescene for Millena SBO can be seen in Figure 42. Fluorescence has a strong wide peak at ex 250-290, em 300-600. Two other peaks can be seen at ex 300, em 400-450 and at em 350, ex 470.

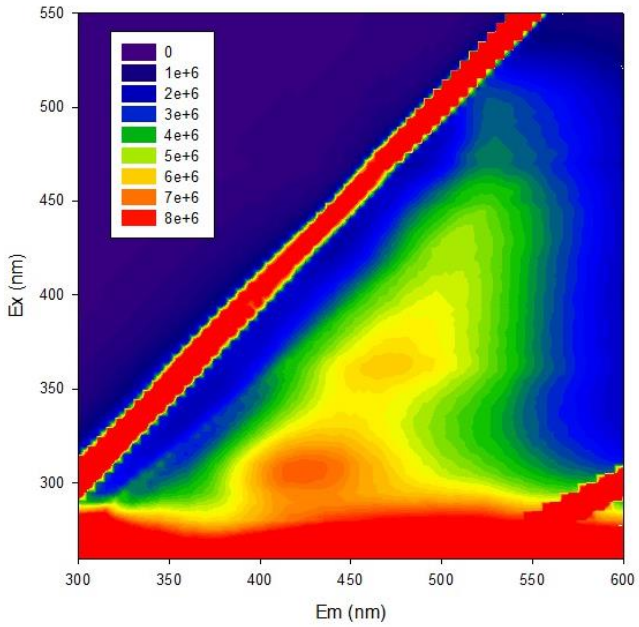


Figure 42 Fluorescence for Millena SBO 1:20

Comparison with the commercial SBO-products show that Millena SBO's fluorescence has a similar shape (Figure 43). Two excitation peaks can be separated from every figure but they are not exactly at the same points than the commercials have. SBO Millena has a wider excitation area on the bottom compared to the commercial SBO-products. This area is assumed to originate from humic-like substances.

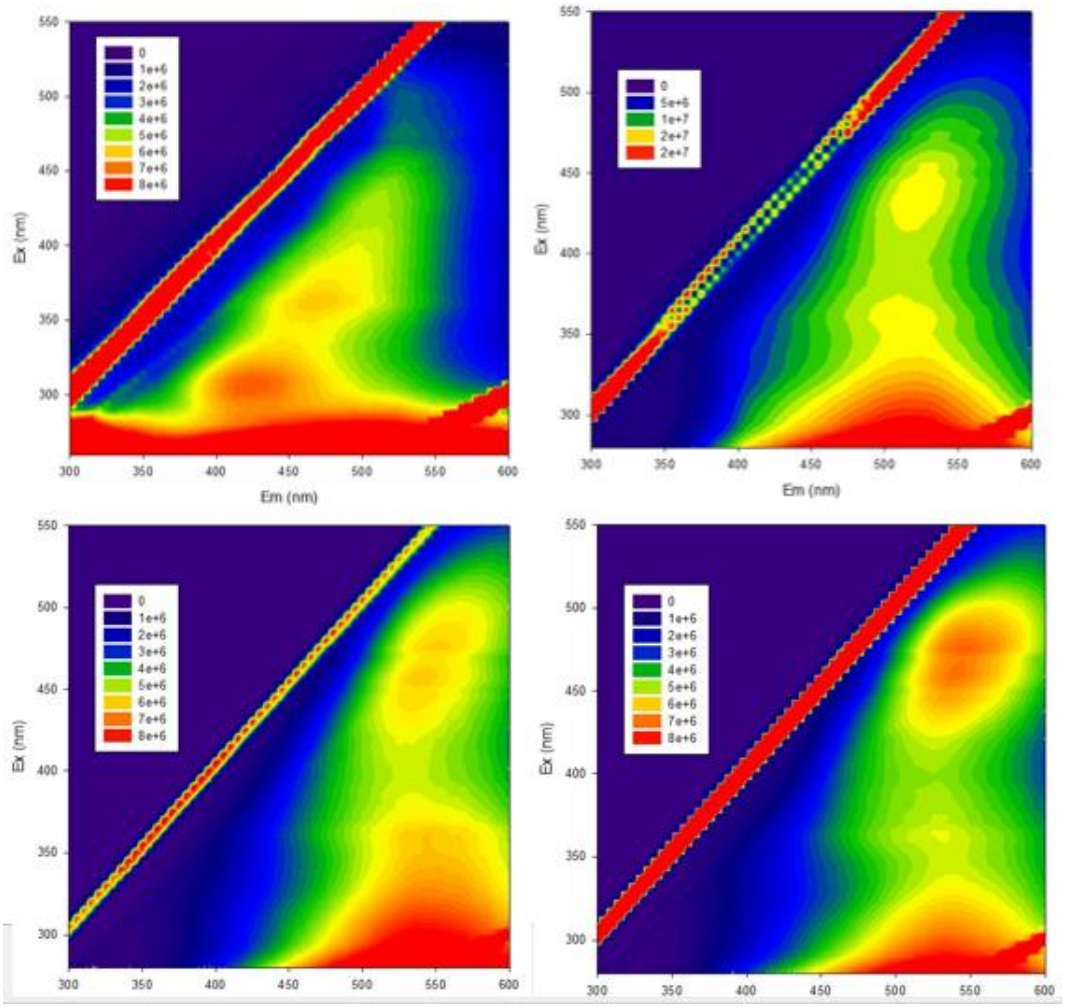


Figure 43 Fluorescenes for Millena SBO (upper left), humic acid sodium salt by Aldrich (upper right), Pahokee peat humic acid (down left) and Leonardite humic acid (down right)

Millena SBO's carbon and nitrogen contents were analyzed with TOC/TN analyzer (Table 7). Pahokee and Leonardite humic acids were used as reference results. According to the results Millena SBO contains more carbon than Pahokee and Leonardite humic acids. This means that Millena SBO probably contains bigger molecules. The shares of organic and inorganic carbons are opposite from commercial products. Millena SBO's bulk of carbon content is organic (78,35%) whereas commercial products contain more inorganic carbon (Pahokee 57,27%, Leonardite 56,9%). Millena SBO's nitrogen content is bigger than the commercial ones.

Table 7 TOC/TN results for Millena SBO, Pahokee peat humic acid and Leonardite humic acid

Sample	Total organic carbon (TOC)	Total carbon (TC)	Inorganic carbon (IC)	Total nitrogen (TN)
Millena SBO 1:20	62,27	79,48	17,22	3,09
Pahokee 1:20	24,35	56,98	32,63	1,97
Leonardite 1:20	24,91	57,79	32,88	0,76

Liquid chromatograph results for Millena SBO are presented as graphs in Figure 44 and numbers in Table 8. The result is different from the earlier ones because now an another peak is shown at retention time 3,26 min. Peak's area is small compared with the humic peak which is at retention time 12.45. The smaller peak has probably formed because of the oven treatment.

Table 8 Liquid chromatograph results for Millena SBO 1:20

Sample	Retention time	Area
Millena SBO 1:20	3,26	25573
	12,45	1053318

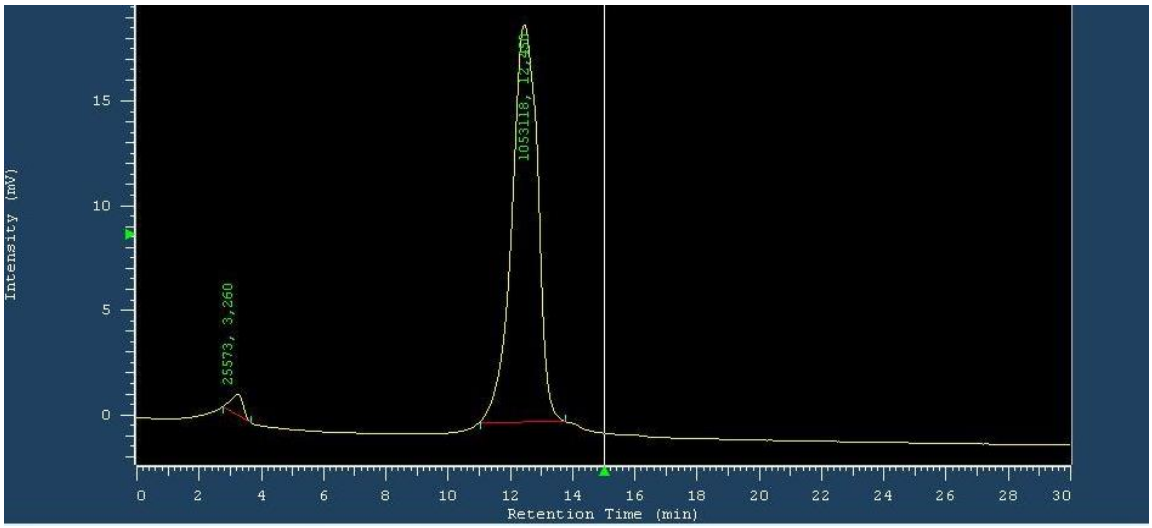


Figure 44 Yellow: Millena SBO 1:20

Compared to the commercial humic products Millena SBO's graph looks very similar with Pahokee peat humic acid (Figure 45). Its retention time is typical for humic substances and the concentration (area) is bigger than the commercial products have.

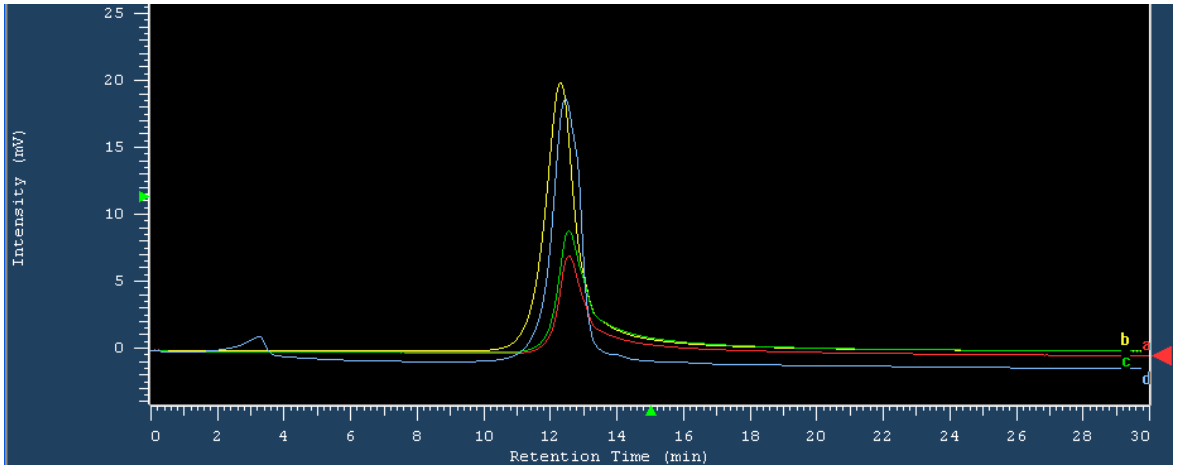


Figure 45 Blue: Millena SBO Yellow: Pahokee humic acid, Green: Leonardite humic acid, Red: Humic sodium salt by Aldrich

5.2 Results of photo-Fenton reaction

Results of photo-Fenton reaction contain only 5 contaminants because an error occurred with amoxicillin's results. It was decided that the results for

5 contaminants offer enough information about the function of the product in this moment of the research. The problem with amoxicillin is probably studied more in the future.

The results for the photo-Fenton reaction performed with Millena SBO are presented in Figure 46 as a plot of the relative concentration (C/C0) vs. time (t(min)). The pH of the photo-Fenton reaction was 5. Graphs start from the point t=-10, C/C0=1 which is the concentration of the solution containing only the contaminants. Second zero t=0 is analyzed from the solution containing the contaminants, Fe and Millena SBO. During the overall 90 minutes reaction time, the contaminants were successfully removed from the solution meaning that iron ions were kept in the reaction by Millena SBO despite the neutral pH. The reaction made without Millena SBO removed the contaminants too but the degradation times were longer (Figure 47). The pH of this reaction didn't stay neutral and this is probably affecting to the results. Normally photo-Fenton reaction requires pH 2-3,5 to function.

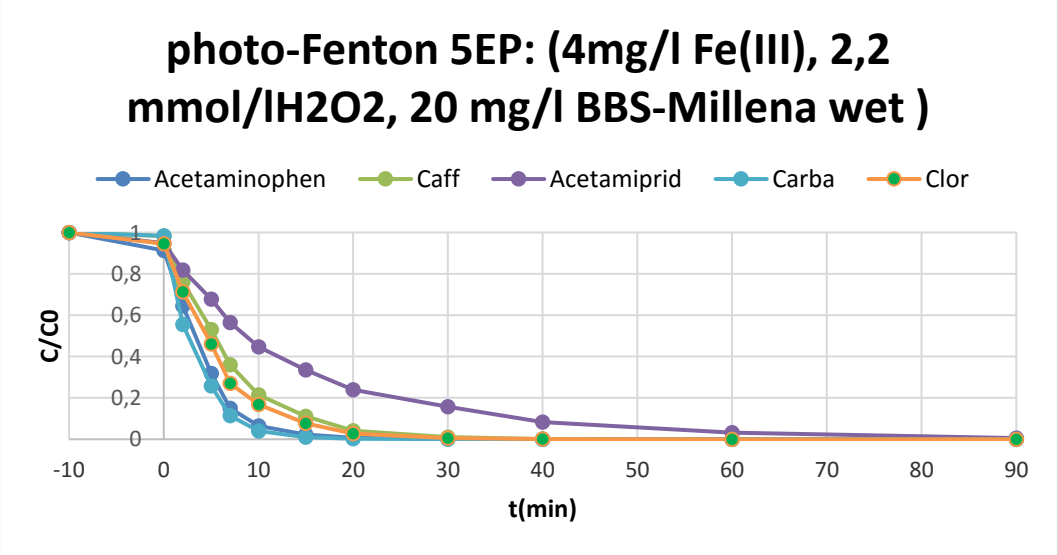


Figure 46 Photo-Fenton results for Millena SBO

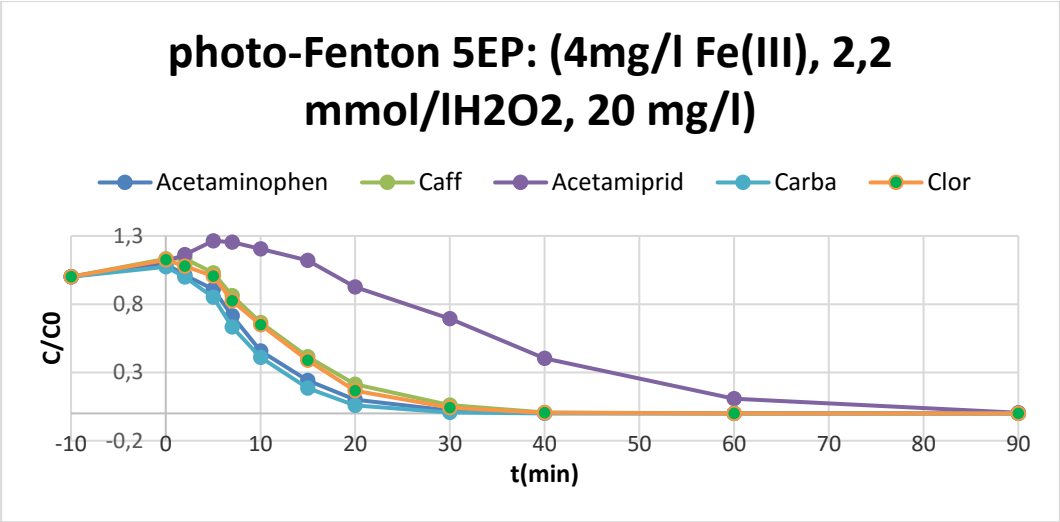


Figure 47 Photo-Fenton results without Millena SBO

Times required to degrade each contaminant to 50% are presented in Table 9. Table contains also the times required when Millena SBO was not used in the reaction. Carbamazepine was degrading most quickly (t=2,56 min) whereas acetamiprid required 8,63 min to degrade to 50%. However the times with Millena SBO are much shorter compared to the reaction without Millena SBO. The biggest difference between the reactions can be seen in acetamiprid's degradation time – without Millena SBO it required 36,69 min while the reaction with SBO got it to degrade in 8,63 min.

Table 9 Illumination time required to degrade each pollutant to 50% of its initial concentration, reaction made with SBO Millena vs. without Millena SBO

t50%		
	Millena SBO	Without Millena SBO
Acetaminophen	3,33	9,50
Caffeine	5,36	13,29
Acetamiprid	8,63	36,69
Carbamazepine	2,56	8,78
Clofibric acid	4,54	17,14

6 DISCUSSION

This study was inspired by the research done in the University of Torino where an SBO-product was successfully made from urban bio wastes using certain digestion and oven treatment. The goal of this study was to find out if hazardous olive oil milling waste called alperujo is suitable for the same purpose by using the same methods they used in Italy.

After the results Millena SBO successfully removed the pollutants in pH 5. Decreasion times were much shorter than with the reaction performed without SBO in first neutral then acidic pH.

This project was the first to try if alperujo is suitable to be a new SBO-product so it was very succesful. One problem occurred with photo-Fenton reaction when there were some problems with the results of amoxicillin. At first amoxicillin did not appear at all in the contaminant solution and afterwards its results were not reliable. It was decided that the results with 5 contaminants offer enough information at this point of the research. At first there were some difficulties with other pollutants too when they were kept too long times at the solution in the fridge before the reaction started and they had dissolved throughoutly. A couple of reactions were performed before the final one. These problems point out that this project was all about experimenting, the research will be easier in the future when the methods etc. are known.

After this project alperujo seems to be a very suitable material to be an SBO-product. It is worth to continue the research with this subject. More studies can be done by trying different digestion methods with different temperatures, pH and times. For example bain-marie can be tested as a digestion environment. It is also worth trying the methods with alperujos from different olive oil milling factories from different areas of Spain. Perhaps soil and climate can have an effect to the composition and quality of the final product. If SBO is used in waste water treatment industry, it requires huge amounts of SBO. After this, it is useful to try to produce big amounts of SBO at the same time. This will be worth to study after the

best method to produce SBO is figured out.

Overall alperujo functions very well as a chemical auxiliary in the photo-Fenton reaction. It is a new way to dispose a hazardous waste besides the other known methods and make the reaction itself more environmentally friendly. Using wastes widely for different purposes along the energy production is an interesting field of study and one step ahead towards a more sustainable world.

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